Efficacy of deltamethrin against ticks on goats in Makhuduthamaga municipality and detection of *Ehrlichia ruminantium* in *Amblyomma hebraeum*

by

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in the

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Declaration

I, Mashifane Mamaje Kgaogelo hereby declare that this dissertation which I hereby submit for the Master of Science degree in the Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, to be my own work and that it has not been previously submitted by me for degree purposes at another tertiary institution.

Mashifane Mamaje Kgaogelo

31 October 2019

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AgriSETA	Agriculture Sector Education and Training Authority
AHT	Animal health technician
Bp	Base pairs
CAHT	Control animal health technician
DAFF	Department of Agriculture, Forestry and Fisheries
DMSO	dimethylsulphoxide
DNA	Deoxyribonucleic acid
DVTD	Department of Veterinary Tropical Diseases
GUTS	Ground-up-tick-supernatant
HWSETA	Health Welfare Sector Education and Training Authority
LDARD	Limpopo Department of Agriculture and Rural Development
MLM	Makhuduthamaga Local Municipality
MLST	Multi locus sequence typing
PCR	Polymerase chain reaction
SV	State veterinarian
SPGB	Sucrose-phosphate-glutamate-buffer
TAE buffer	Tris-acetate-EDTA buffer

UCTD	Utrecht Centre for Tick-borne Diseases

Summary

Efficacy of deltamethrin against ticks on goats in Makhuduthamaga municipality and detection of *Ehrlichia ruminantium* in *Amblyomma hebraeum*

By

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Degree: MSc Veterinary Science Tropical Diseases

Department: Veterinary Tropical Diseases

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Key words: *Ehrlichia ruminantium*, *Amblyomma hebraeum*, heartwater, ticks, goats, deltamethrin, acaricidal efficacy, Makhuduthamaga

The aim of this study was to develop an improved strategy for the control of tick infestation on indigenous goats owned by emerging farmers in Limpopo Province, South Africa. The acaricidal efficacy was determined using a pour-on deltamethrin treatment on goats infested by ticks in Makhuduthamaga rural areas. The study was conducted between February and July 2018. In total, five livestock owners in three villages were involved in the study. Goats were divided into two groups: treated and untreated control groups, each consisting of five goats. Goats in the treatment group were treated with the pour-on acaricide deltamethrin Clout®, whereas the untreated goats from within the same household served as controls. Topical treatment with pour-on deltamethrin (Clout®) reduced tick numbers by 94.5% on indigenous goats in the treatment group compared to the control group. In total, 5,671 ticks were collected from the control group, with average of 9.44 ticks per goat, whereas a total of 296 ticks were collected from the treated group, with an average of 0.52 ticks per goat. Furthermore, it was observed that the number of ticks counted on the control goats slowly

declined during the trial, which coincided with the approach of seasonal conditions that are less favourable for ticks. Reducing the acaricidal application interval to two weeks was recommended, since ticks were already re-infesting the goats toward the end of the application interval of three weeks. Lameness of goats was often observed due to massive infestation of ticks on the feet of control goats.

The study also contributed to the process of isolating current strains of heartwater to further our understanding of the disease dynamics and pathogenicity for small ruminants. *Ehrlichia ruminantium*, the causal agent of heartwater disease in ruminants, was detected in field collected *Amblyomma hebraeum* ticks and cryopreserved as ground-up-tick-supernatants (GUTS). One of the 8 GUTS (12.5%) was found to be positive for *E. ruminantium* by pCS20 PCR. Ground-up-tick-supernatants need to be tested for their infectivity to induce heartwater in experimental goats and adapted to *in vitro* growth in endothelial cell cultures to broaden the array of currently available heartwater strains. Including goats in strategic tick control programmes is recommended in order to reduce lameness due to *A. hebraeum* infestation and the risk of contracting heartwater disease.

Introduction

Previous research conducted in the Mnisi Community Area in Mpumalanga Province, South Africa has demonstrated that *Amblyomma hebraeum* is the predominant ixodid tick, infesting indigenous goats owned by emerging farmers (JONGEJAN et al., submitted for publication). Skin lesions, in particular in the inter-digital space (a predilection site for the ticks) were shown to be characterised by secondary infections and lameness. Questionnaire surveys conducted in the households situated in the Mnisi area indicated that over 80% of owners had seen lameness due to tick infestation on their goats (JONGEJAN et al, submitted for publication). Moreover, the majority of owners responded that they had lost one or more goats in the preceding year. These goats had either died suddenly or displayed leg-pedalling movements prior to death. A definite diagnosis was not made, however, because goats are a primary source of meat and therefore not usually submitted for post-mortem diagnosis.

Despite the importance of goats for the livelihoods of farmers in the area, tick control is not practiced on any systematic scale, due to a lack of education and resources. However, at one of farmer's day held in Mnisi area, clear interest was shown by the owners for improved animal health management through tick control on their goats, leading to their enhanced livelihood. As a result, and after consultation with the local authorities and farmer's association, a number of interventions were carried out with the aim of reducing the direct impact of long mouthparts species like those in genus *Amblyomma* on the health of indigenous goats owned by emerging farmers in the Mnisi area. One of the interventions in Mnisi concerned the application of acaricides at monthly intervals on selected groups of 5 goats per household. In the weeks after treatment, a dramatic decrease in tick numbers was observed in the treatment group. This was the reason for studying a similar approach in other areas of South Africa, where small-holder farmers are facing the same difficulties with respect to *Amblyomma* ticks.

Here, an efficacy trial was conducted using pour-on deltamethrin on goats belonging to households in the Makhuduthamaga rural areas of Sekhukhune district in Limpopo province, South Africa.

Amblyomma ticks are also important with respect to their capacity to transmit *Ehrlichia ruminantium,* which is the causal agent of heartwater. *Ehrlichia ruminantium* is an intracellular rickettsia belonging to the family of the Anaplasmataceae (ALLSOPP, 2015). Heartwater is one of the most economically important tick-borne diseases in South Africa, where it affects improved (*Bos taurus*) breeds of cattle, sheep and certain breeds of goats such as angora. In general, *E. ruminantium* infection may cause clinical signs depending on the pathogenicity of the genotype, but also on the immune status of the host, breed and age. Small ruminants are at particular risk, and may die suddenly or displaying leg-pedalling movements prior to death. Heartwater has been shown to be responsible for the gradual reduction of commercial sheep farming in Limpopo Province (BRYSON et al, 2002). The individual treatment of heartwater cases is often undertaken too late and tick control is not usually strictly administered to completely prevent the transmission of *E. ruminantium* by infected *A. hebraeum* ticks (YUNKER, 1996).

Ehrlichia ruminantium has been isolated and grown *in vitro* in endothelial cell cultures, which have been used as the basis for genome projects. The first such genome of *E. ruminantium* sequenced was based on a South African isolate, and other isolates followed thereafter. However, if genetic recombination takes place in the field, strains that have been isolated decades ago, such as the Ball 3 strain, may no longer exist. Therefore, there is a need to isolate current strains of heartwater from the field. The isolation of current strains and their comparison with historical isolates will facilitate our understanding of the disease dynamics and ultimately lead to improved disease control.

It was predicted that deltamethrin would dramatically reduce the tick burden on treated goats and indirect damage due to ticks (lameness) would be minimised, leading to improved animal health and wellbeing. Moreover, deltamethrin-treated goats are expected to have a lower risk of succumbing to heartwater in comparison with non-treated control goats, since the challenge with infected ticks is much lower. A significant positive effect may be measurable on the livelihoods of the participating farmers and the study model can be adopted by other farmers once the benefits have been demonstrated.

Aim

The ultimate aim of this study was to contribute to an improved strategy for the control of tick infestation on indigenous goats owned by emerging farmers in Limpopo Province, South Africa.

Objectives

Determine the acaricidal efficacy of deltamethrin (Clout®) on indigenous goats against ticks with particular reference to *A. hebraeum* and the detection of *E. ruminantium*.

Hypotheses

1. Topical treatment with a deltamethrin pour-on solution will reduce tick numbers on indigenous goats in the treatment group compared to the control group.

2. *Ehrlichia ruminantium* can be detected in field-collected *A. hebraeum* ticks and cryopreserved as ground-up-tick-supernatants.

Ethical clearance and study permits

Ethical clearance was obtained from the University of Pretoria Ethics Committee (Project number V119-17). The participants were provided with an informed consent form obtained from the same ethics committee and field trials were commenced only after farmers had completed the forms (See Appendix B).

Permission under section 20 of the Animal Diseases Act, 1984 (Act No 35 of 1984) to perform research/study

The permission to conduct the research project was obtained from the Department of Agriculture, Forestry and Fisheries for the transportation of ticks from Limpopo Province at Makhuduthamaga rural areas, Sekhukhune district to Gauteng province to be used in Onderstepoort at the laboratories of the Department of Veterinary Tropical Diseases of the University of Pretoria. Permission was also obtained from the State Veterinarian from Limpopo Department of Agriculture and Rural Development to conduct this research study with Makhuduthamaga small stock communal farmers according to section 20 of the Animal Diseases Act 35 of 1984. Finally, ethical clearance and all study permit approval documents are also attached (See Appendix C).

Literature review

Amblyomma hebraeum ticks

Ixodid ticks, in particular *Amblyomma* species, are of significant economic importance as vectors of livestock diseases in Southern Africa (NYANGIWE & HORAK, 2007). *Amblyomma hebraeum,* also known as the South African bont tick, is abundant in Southern Africa in climatic regions, varying from tropical to more arid areas. *A. hebraeum* infests cattle, sheep and goats, as well as a range of wildlife species (WALKER et al., 2014). The tick is present in a number of southern African countries, such as Swaziland, Zimbabwe, Botswana and Mozambique, as well as South Africa (JONGEJAN & UILENBERG, 2018). In South Africa, *A. hebraeum* is widely distributed along the coastal belt from Port Elizabeth in the Eastern Cape Province, through Kwazulu-Natal and across Mpumalanga, Gauteng to Limpopo province (Figure 1).

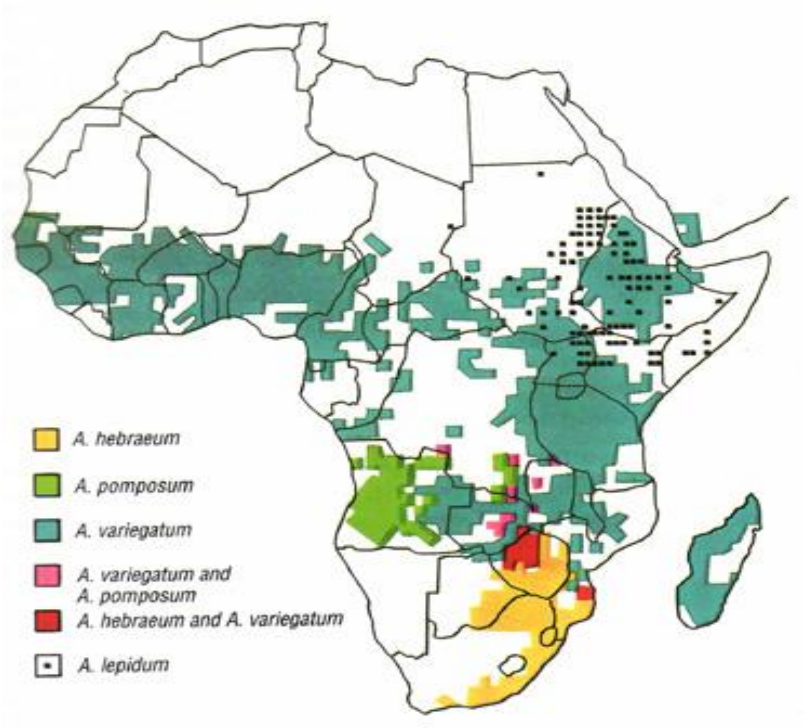

Figure. 1a. Distribution of *Amblyomma* ticks in Sub-Saharan Africa (www.afrivip.org)

Amblyomma hebraeum is a member of family of the Ixodidae, characterised by a hard sclerotized plate on the dorsal body surface and have banded legs, long mouth parts and are bigger than most other tick species. Moreover, the scutum is ornate and the eyes are flat. Scutum in males covers the entire dorsal body surface and carries many small setae. *A. hebraeum* is a three-host tick, consisting of different developmental stages, larvae, nymphs and adults, which are regarded as hunter ticks actively seeking their hosts. The engorged females oviposit large batches of approximately 20 000 eggs after they have detached from their hosts (MADDER et al., 2011). The eggs hatch into larvae, which remain in the vegetation awaiting the host. Larvae feed on the host and moult in the vegetation into nymphs. The nymphs feed again on another host and thereafter moult into either an adult female or a male.

Interestingly, adult males attach first and start producing pheromones while feeding to attract other males, females and nymphs, making tick-infested hosts more attractive to ticks in the vegetation. Males and females copulate and the females engorge over a period of 7-9 days and then drop from their host. Males can remain attached to the same host for several months, during which period they continue to produce pheromones to attract other ticks (JONGEJAN & UILENBERG, 2018).

In South Africa, ticks are less prevalent during colder and dry autumn and winter months compared to spring and summer during the warm and wetter season. However, low varying numbers of all developmental stages can be found on the hosts all year round. This tick is not found in the highveld areas of South Africa, whereas it may be restricted by interspecific competition with *A. variegatum* in central Mozambique and northern Zimbabwe. In addition, because of its long mouthparts, it can cause secondary infections, whereby the blowfly *Chrysomya bezziana* can be attracted to the lesions with myiasis as the result.

Tick control

In general, it is important to control ticks and tick-borne diseases across those regions where ticks are a challenge in order to reduce the impact on livestock production. The prolonged feeding period of adult ticks allows treatment with acaricides at two-weekly intervals. Since *A. hebraeum* predominantly prefers hairless areas in the lower perennial region, at the axillae, genitalia, on the udder and under the tail of cattle, as well as attaching to the inter-digital space of goats and sheep, control may be more effective by targeted application of acaricides (MADDER et al., 2011).

Ehrlichia ruminantium infection

Amblyomma ticks transmit *E. ruminantium,* the causal agent of heartwater. *E. ruminantium* belongs to the family of the Anaplasmataceae and is widely distributed throughout sub-Saharan Africa (ALLSOPP, 2015). Heartwater is a disease affecting domestic ruminants, in particular improved cattle breeds and small ruminants. It has been known for decades that heartwater strains historically originate in the province of Limpopo, where the disease was first noticed by Louis Trichardt while trekking through with his flocks of sheep in 1838 (YUNKER, 1996).

The relative abundance and seasonal dynamics of *Amblyomma* ticks and the prevalence of infection with *E. ruminantium* are important factors to take into account when studying the epidemiology of heartwater (JONGEJAN & UILENBERG, 2018). This disease has extended its distribution through the movement of tick-infested livestock into the Caribbean, from where it constitutes a threat to the American mainland (ALLSOPP, 2015). Moreover, trade and movement of livestock across geographical regions jeopardise the establishment of a robust immunity against heartwater, for instance in restocking exercises in Mozambique (BEKKER et al., 2001). These challenges affect livestock farming in heartwater endemic areas.

Heartwater affects different species of livestock, cattle, sheep and goats, as well as some susceptible wild antelope species. However, small ruminants appear to be the predominantly

affected species (YUNKER, 1996). Disease symptoms include high fever, nervous signs and the accumulation of fluid in the lungs, brain, thoracic cavity and pericardial sac. The transmission of heartwater to a susceptible goat often results in an acute fatal disease. In endemic areas, the prevalence of heartwater is often under-reported, because a definitive diagnosis is often not made. In particular, goats are affected. It is unclear what the actual mortality due to heartwater is, but this is nevertheless considered significant. Certain breeds of goats are more resistant than others, making the introduction of high-producing animals into rural *Amblyomma* areas difficult.

The control of heartwater is targeted towards the therapeutic treatment of clinical cases in combination with acaricides for tick control. Immunity established either through natural exposure of young animals, while possessing an innate resistance to the disease, or by using a heartwater blood-based vaccine has been practised to some degree. However, failure of the Ball-3 blood-based vaccine strain of heartwater to confer cross protection to immunologically different strains has been demonstrated (JONGEJAN et al., 1991). Vaccines based on the Ball-3 isolate are available, but are not used in the traditional livestock sector. Attenuated vaccines have been developed (JONGEJAN et al., 1991; ZWEYGARTH et al., 2005; 2008), but are not yet commercially available in South Africa. Novel approaches include recombinant vaccine development based on the MAP1 protein (FABURAY et al., 2017).

Genotyping of *E. ruminantium* with the aim of mapping genetic diversity has provided evidence that genetic recombination is a major driving force of this diversity (ALLSOPP & ALLSOPP, 2007; CANGI et al., 2016). Such studies have used ticks collected from livestock in heartwater endemic areas for the DNA extraction of *E. ruminantium*, which were subsequently characterised by different genotyping methods, such as multi locus sequence typing (MLST) (NAKAO et al., 2011).

If one wants to go beyond the sequencing of a limited number of genes, whole genome sequencing is the next step, but this requires larger amounts of purified DNA. For this purpose, *E. ruminantium* has been isolated and grown *in vitro* in endothelial cell cultures, which has been used as the basis for genome projects. The first such sequenced genome of *E. ruminantium* was based on a South African isolate (COLLINS et al., 2005). Recently, three

isolates from Zimbabwe, the Gambia and Ghana were sequenced because sufficient amounts of material grown *in vitro* and purified was available (NAKAO et al., 2016).

In order to perform genetic typing of infected ticks, they have to be fixed in ethanol, and thereafter it is no longer possible to isolate live *E. ruminantium* organisms. However, if ticks are kept alive, they can be used to isolate *E. ruminantium* strains, which will make it possible to correlate the genotype with the actual phenotypic behaviour of isolated strains in terms of virulence for animals and, more importantly, whether they afford any cross-protection. If genetic recombination takes place in the field, then those strains that were isolated decades ago, such as the Ball-3 strain, may no longer exist. As a consequence, if for instance novel vaccines are to be evaluated, it is essential to do this against current field strains.

Therefore, the isolation of current strains and their comparison with historical isolates can facilitate our understanding of disease dynamics, ultimately leading to improved disease control.

Materials and Methods

Efficacy trial of deltamethrin (Clout®) treatment against ticks on goats

Study area

The study was conducted between February and July 2018 at Makhuduthamaga rural areas in Sekhukhune district of Limpopo province, South Africa (See Appendix A). Makhuduthamaga local municipality is located ±189 km south east of Polokwane, ±285 km south west of Kruger National Park, ±247 km north east of Pretoria and ±347 km north east of Johannesburg. It is situated in an economically depressed area, and, consequently, the municipality has indicated in annual reports that it has difficulties in attracting and retaining skilled professionals and are struggling from a revenue generation perspective (MLM, 2012; MLM,2019)

The area is entirely rural in nature with communal tenure, dominated by custodianship of local traditional authorities in terms of land ownership. Most of the land is used for subsistence purposes, with only a small portion of the land under commercial farming. The municipality consists of a land area of approximately ± 2,096 square metres and made up of ± 189 settlements with a population of ± 274,358 people and ± 65,217 households, of which ±1,799 are communal goat farmers (MLM, 2019).

Although the climatic conditions are highly variable in terms of rainfall intensity, duration and frequency, they are characterised by a hot, fairly dry (semi-arid) climate as part of the Olifantsriver valley. Moreover, the area is susceptible to extreme climate conditions which can oscillate between floods and droughts. The average rainfall is between 500 and 800 mm annually, with more than 80% of its rainfall occurring between September and March, at times extending to April. Thunderstorms with the associated low soil penetration and high level of erosion are common in the area. The average summer temperatures are 23°C, with a mean maximum value of 28°C and a mean minimum of 18°C. In winter, the average is 13.5°C with a maximum of 20°C and a minimum of 7°C. The topography varies between flat and undulating slopes interrupted by koppies, steep slopes that makes the area prone to erosion.

Makhuduthamaga rural areas are mostly tropical bush, savannah, which is classified as Sekhukhune Plains Bushveld (MUCINA & RUTHERFORD, 2006)

Study animals

Four villages, namely, Phokwane, Mabintwane, Maserumo and Kutupu were randomly chosen for the study. However, Kutupu village was removed prior to the start of the field trial due to the high stock theft reported. Two households were selected in each of the villages Phokoane and Mabintwane, whereas one household was selected in the village of Maserumo. Thus, in total, five livestock owners in three villages were involved in the study. Distances between villages were such that they could be reached by bicycle within a reasonable time. In each household, ten healthy adult medium-sized (35-70 kg) goats with short hair (excluding males that were capable to mate) were selected randomly to be included in the study. In total, there were 50 goats sampled: 25 goats in the treatment group and 25 goats which served as untreated group.

Acaricidal treatment

Goats were divided into two groups, treated and untreated groups, each consisting of five goats. Goats in the treatment group were treated with the pour-on acaricide deltamethrin Clout®, whereas the untreated goats from within the same household served as control groups. Both groups were kept in separate kraals at night to avoid the transfer of acaricide between goats belonging to different groups, but were free ranging together during the day.

Prior to the start, all goats were screened for the presence of ticks and ticks were removed on day zero. Thereafter, on day zero and thereafter every 21st day, the acaricide was applied to goats in the treatment group. Evaluation was carried out by counting ticks *in situ* and then seven days after each acaricidal application. Goats in the control group were also monitored for ticks every seventh day.

The duration of the efficacy trial was 168 days (5 months and two weeks), wherein 8 treatments of goats with acaricide Clout® were conducted at three weekly intervals (every 21 days). The observation period of deltamethrin against ticks in the treated group was 36 days

and untreated group was also observed during the same period. In addition, the administration of acaricide deltamethrin together with tick counting was carried out in the last trimester of summer, the 3 months of autumn and the first 2 months of winter in 2018.

The goats in the treatment group were weighed using a goat-weighing-belt before being treated with the pour-on acaricide. The measured dose was 1 ml per 5 kg as per manufacturer's instruction and the calculated dose was applied on selected goats using a 10ml syringe along the back, neck, and between the horns, partitioning the hair coat on the skin, as well as towards the predilection side (at axillae, genitalia, perennial region, under tail, udder and between the hoofs) of *A. hebraeum* and other tick species.

The treated groups were monitored for the efficacy of the acaricide on every 7^{th} day. In the untreated control, the selected goats were also examined for the presence of *A. hebraeum* and other ticks on the days stipulated above. This implies that a different household was visited on each day of the week.

Questionnaires of small holder goat owners on current tick control practices

The questionnaire forms were used to acquire current and previous information about the goats. Any abnormality, for instance lameness, was recorded using the data capture recording form (See Appendix D).

All ethical procedures were enforced when handling and restraining the goats with proper consideration for their welfare.

The relative proportion of *A. hebraeum* ticks present on goats

Tick counting

The treated and untreated groups were monitored on every day 7th for the number of live or dead *A. hebraeum* and other tick species present and their predilection site was recorded. Where ticks were found, they were counted and removed to avoid recounting. Only nymphs and adult ticks were counted.

The standardised method of tick counting was implemented during the inspection of ticks at the predilection site and on the goat's body (See Appendix E). The different household were visited on each day of the week.

Efficacy of the deltamethrin Clout® against *Amblyomma hebraeum* and other ticks' species

Tick counting was performed for live and dead ticks in both the treatment and control groups; the data was captured using Excel spread-sheet (Appendix E). Data was then interpreted and graphs were developed. This was done in order to evaluate the efficacy of deltamethrin Clout® against all ticks, as well as against *A. hebraeum* ticks only.

The efficacy of the treatment was determined using the following formula:

Efficacy (%) = 100 x (C – T) / C, wherein:

C = Mean tick count on the control group; T = Mean tick count on the treated group.

(HOLDSWORTH et al., 2006)

Collection of live *A. hebraeum* adult ticks for ground-up-tick-supernatants (GUTS)

Approximately 50 *A. hebraeum* ticks were collected from untreated goats not enrolled in the study but which belonged to the same households that were involved in the study. Ticks were stored in small red-lidded bottles and labelled with the date of collection and owners' name, with coordinates, the site of collection, number of goats sampled and number of ticks collected with sufficient ventilation.

Immediately after collection, the ticks were transported to the University of Pretoria in a cool bag to keep them alive until further processing. Collections started on day 0 and thereafter every 21 days, resulting in eight batches of ticks.

Molecular detection of *E. ruminantium* in *A. hebraeum* ticks

Ground-up-tick-supernatant (GUTS) were based on approximately 400 *A. hebraeum* ticks. Upon arrival, the ticks were treated immediately by rinsing them in tap water and allow to air dry. Thereafter, ticks were triturated in a mortar filled with sucrose-phosphate-glutamate buffer (SPGB) on ice. The resulting GUTS was allowed to stand in a 10ml tube for 15 min on ice in order to sediment the separate parts. Thereafter, dimethylsulphoxide (DMSO) was slowly added on ice until a 10% end volume was reached. The GUTS were then divided into 1ml cryo-tubes and frozen in dry ice at -70°C, before being placed in a -20°C freezer for temporary storage. The positive GUTS were stored in a freezer at -140°C for long-term storage at the University of Pretoria laboratories at the Department of Veterinary Tropical Diseases (DVTD). This was done in order to make new isolates available in storage, in case genetic recombination takes place in the field. An aliquot was spotted onto FTA cards in order to bind the DNA to facilitate easy transport and subsequent PCR testing.

Extraction of genomic DNA

Total genomic DNA was extracted from FTA cards using QIAamp DNA Mini Kit (Qiagen) following the manufacturer's instructions.

The FTA cards were cut into small pieces in a mixture of 200 µl lysis buffer (ATL), 20 µl proteinase K and mixed by vortexing. The mixture was incubated at 56°C for 10 minutes. Two hundred (200) µl Buffer AL was added to the suspension, mixed and incubated at 70°C for 10 minutes. Two hundred (200) µl ethanol (96%-100%) was added to the sample, mixed and transferred to a QIAamp Mini Spin column in a 2 ml collection tube. The sample mixture was centrifuged at 8000 rpm for 1 minute. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. Five hundred (500) µl Buffer AW1 was added to the QIAamp Mini spin column and centrifuged at 8000 rpm for 1 minute. The QIAamp Mini spin column was placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. Five hundred (500) µl Buffer AW2 was added to the QIAamp Mini spin column and centrifuged at 14000 rpm for 3 minutes. The QIAamp Mini spin column was then placed in a clean 2 ml collection tube and the tube containing the filtrate was discarded. Two hundred (200) µl Buffer AE was added to the QIAamp Mini spin column, incubated at room temperature for 1 minute and centrifuged at 8000 rpm for 1 minute. The DNA was stored at -20 °C until further use.

Nested PCR targeting pCS20

The purified DNA from the GUTS was analysed for the presence of *E. ruminantium* strains using a nested PCR amplification technique, with both negative and positive PCR controls. The first primer pair utilised with PCR cycling conditions for *Ehrlichia* was AB128dege (5'-CCAGCAGGTAGTGTTTACATTAGCGCA-3') with the reverse primer AB30dege (5'-CAAACCTTCCTCCAATTTCTATACC-3'); the second forward primer was the same as above with the reverse primer AB129dege (5'-GGCAAACATCAAGTGTTGCTGATGC-3'). These primers amplify a fragment of the pCS20 gene, which is a target used to map the broad diversity of *E. ruminantium* strains (VAN HEERDEN et al., 2004). They have been successfully used to detect *E. ruminantium* strains in *A. variegatum* ticks collected from small ruminants (FABURAY et al., 2007).

The first round of nested PCR amplification was performed in a 20 µl reaction with 10 µl 2X High-Fidelity PCR Master Mix, 2 µl template DNA, 1 µl forward primer. 1 µl reverse primer and 6 µl distilled water.

The second round was performed in a 20 µl reaction with 10 µl 2X High-Fidelity PCR Master Mix, 0.5 µl template DNA, 1 µl forward primer, 1 µl reverse primer and 7.5 µl distilled water.

The PCR program consisted of: initial denaturation at 98 °C for 10 seconds followed by 30 cycles of denaturation at 98 °C for 1 second, annealing at 50 °C for first PCR and 55 °C for second PCR for 5 seconds and extension at 72 °C for 15 seconds. Final extension was at 72 °C for 1 minute.

Electrophoresis gel and PCR products

The amplified PCR products were analysed by electrophoresis on 1.5 % agarose gel in TAE buffer mixed with 3 µl ethidium bromide. When viewed with UV light illumination, samples were considered positive if exhibiting a 300bp band (CANGI et al., 2016).

Results

Questionnaires of small holder goat owners on current tick control practices

The study site at Makhuduthamaga municipality, Limpopo province is illustrated in Figure 1b.

The survey results indicated that three household goat owners were using non-registered traditional remedies for tick control, whereas the other two owners did not perform any form of control (See Appendix D). Owners emphasised that when their goats fell ill, they were not capable of treating them with any registered remedies due to insufficient funds. One household goat owner stipulated that some of his goats had died after displaying walking in a circle, paddling legs and protrusion of the neck. Four owners highlighted that their goat numbers had reduced due to cannibalism and stock theft. Furthermore, they observed massive infestation of ticks on the feet of their goats and observed lameness, particularly in the summer.

Efficacy trial of deltamethrin (Clout®) treatment against ticks on goats

The ticks were counted on every 7th day in both the treatment and control groups. Most ticks were found attached to the feet, ears, under the tail, and armpits, whereas fewer ticks were found on other body parts. On each day of the week, individual households and goat owners were visited. The number of ticks counted in the deltamethrin Clout® group and untreated control group over a period of 168 days was recorded. The goats were examined for the presence of ticks and were then removed on day 0 in both groups, with deltamethrin Clout® being simultaneously applied on goats in the treatment group. Tick counting was carried out every 7th day, whereas acaricide was applied on goats on every 21st day. The effectiveness of deltamethrin was seen in most cases. In untreated control groups, the tick loads were higher; however, due to the removal strategy, the goats had to be re-infected weekly.

The efficacy of the treatment using the following formula:

Efficacy (%) = 100 x (C – T) / C, wherein:

C = Mean tick count on the control group; T = Mean tick count on the treated group.

A total of 5,671 ticks were found in the control group of 25 goats, with an average number of 236 ticks per time point and 9.44 ticks per goat. On the other hand, in the treatment group of 25 goats, a total of 296 ticks were found, with an average number of 13 ticks per time point and 0.52 ticks per goat (Figure 2; Figure 9). The differences between both groups were significant at all-time points (Mann-Whitney U test).

The relative proportion of *Amblyomma* ticks on these goats versus the total tick numbers was 56% in the treatment group, whereas 12% of the ticks in the control group were *A. hebraeum.* The overall efficacy based on *Amblyomma* ticks only was only 72%. *Amblyomma hebraeum* ticks in the treatment group (25 goats) compared to the control group (25 goats) of five households in three villages at Makhuduthamaga rural areas are shown in Figure 3.

Tick counts on goats per household are given in Figure 4a-b for Mabintwane-B village. Ticks counted on goats in Phokwane Ga-Ramasehla village are shown in Figure 5a-b, tick counts on goats in Mabintwane-A village in Figure 6a-b and those counted in Phokwane village (Ka-7) appear in Figure 7a-b. Finally, ticks that were counted on goats in Maserumo village are shown in Figure 8a-b.

The following irregularities occurred, whereby some of the goats had to be replaced.

*On day 42, two goats from the control group appeared unhealthy and were replaced by reserved goats within the same household.

*On day 49, one goat from the control group and one from the treatment group went missing and did not return from the veld; they were replaced by other goats from the same household goat owner's flock.

*On day 84, while evaluating the efficacy of deltamethrin Clout®, three goats from the treatment group were reported missing due to stock theft during the night while the owner was sleeping. The procedure for observing and counting was only performed on the two remaining goats, while the missing three goats were replaced on the same day with goats from the same household.

* On day 105, the owner sold two goats from the control group and one from the treatment group to secure food, cultural and traditional matters. The goats were replaced by other goats from within the same household.

*On day 147, observations of ticks in both groups and the application of deltamethrin Clout® in the treatment group were difficult due to a village strike that lasted for almost a week. This hampered the procedure as the last day for applying acaricide was day 154. On day 147, there was a strike around the villages; however, the observation, counting and acaricide application was performed due to the fact that the procedures were performed in the late afternoon towards the evening when the strike recessed. In total, 10 goats were replaced during the study.

Figure 1b: Study site: Indigenous goats at Kutupu & Mabintwane, Makhuduthamaga municipality, Limpopo province (April, 2017)

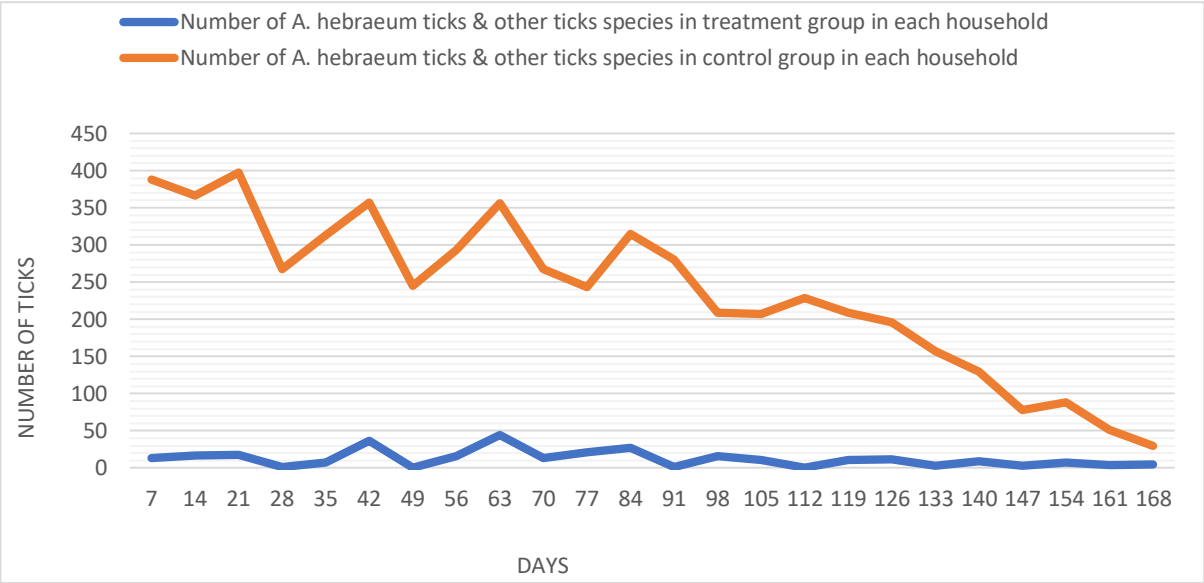

Figure 2. Comparison of the total number of ticks in the treatment (25 goats) and control group (n=25) in five households of three villages at Makhuduthamaga rural areas.

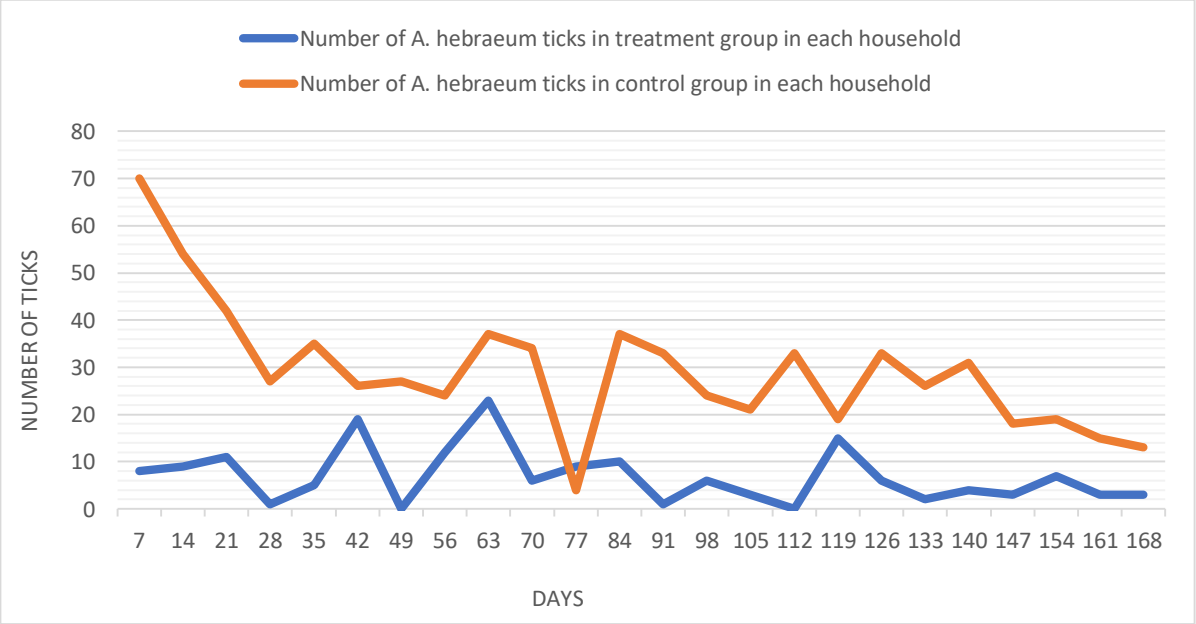

Figure 3. Comparison *A. hebraeum* ticks in the treatment group (25 goats) compared to the control group (25 goats) of five households in three villages at Makhuduthamaga rural areas.

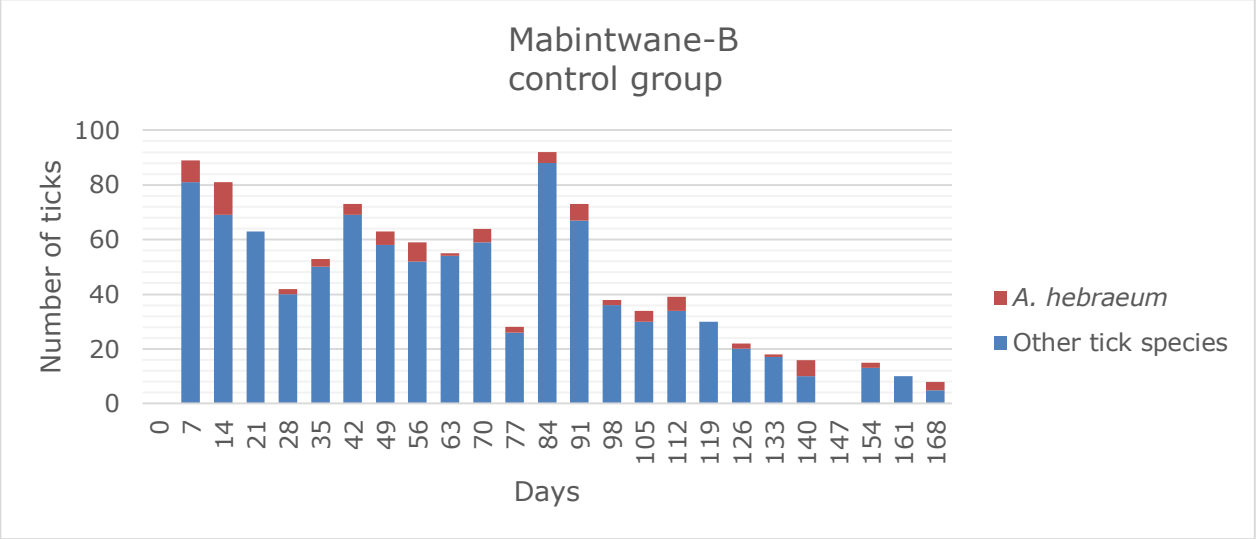

Figure 4a. The total number of *A. hebraeum* and other tick species in a control group of 5 goats in one household at Mabintwane-B village.

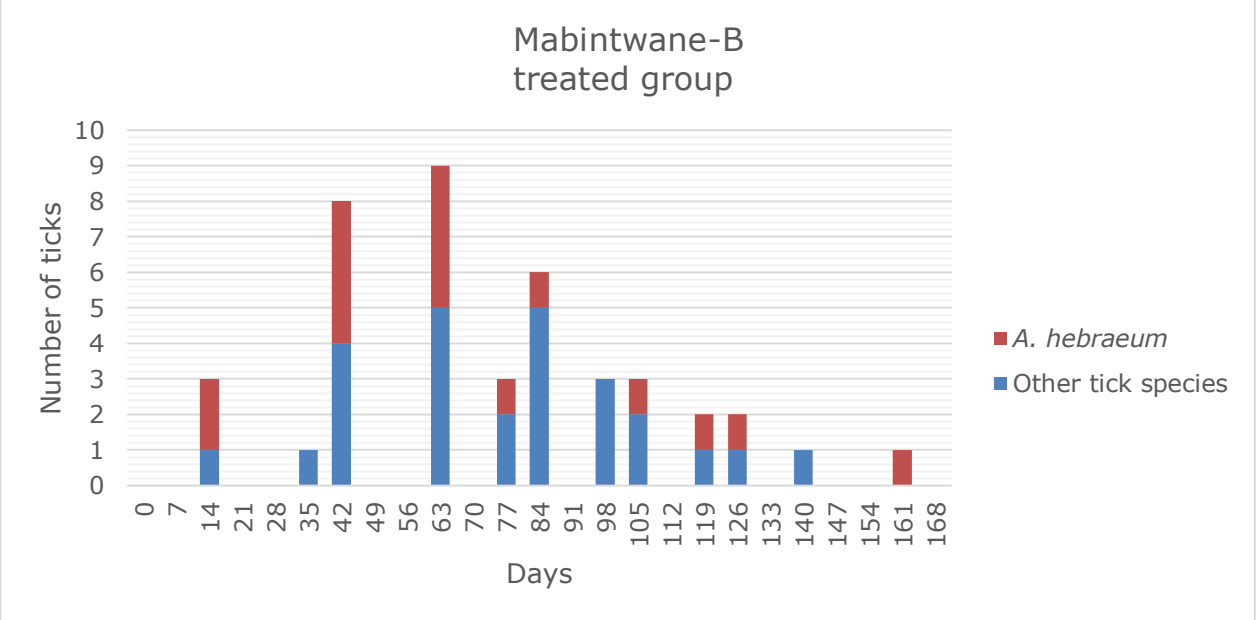

Figure 4b. The total number of *A. hebraeum* and other tick species in a treated group of 5 goats in one household at Mabintwane-B village.

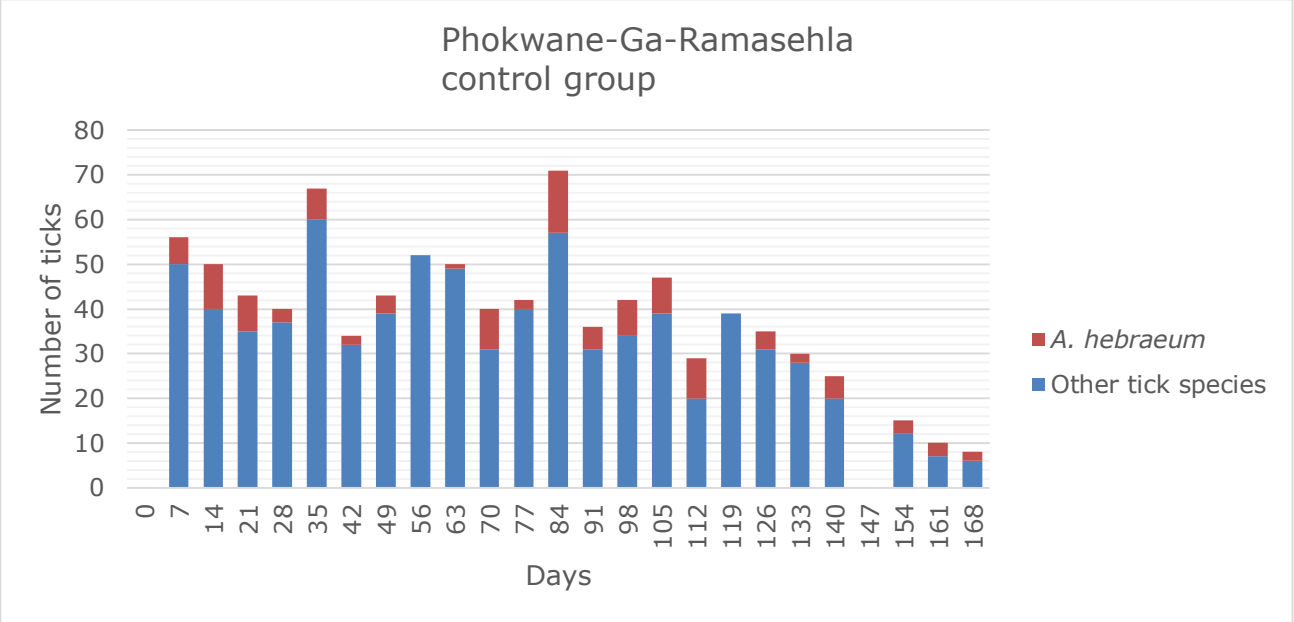

Figure 5a. The total number of *A. hebraeum* and other tick species in a control group of 5 goats in one household at Phokwane Ga-Ramasehla village.

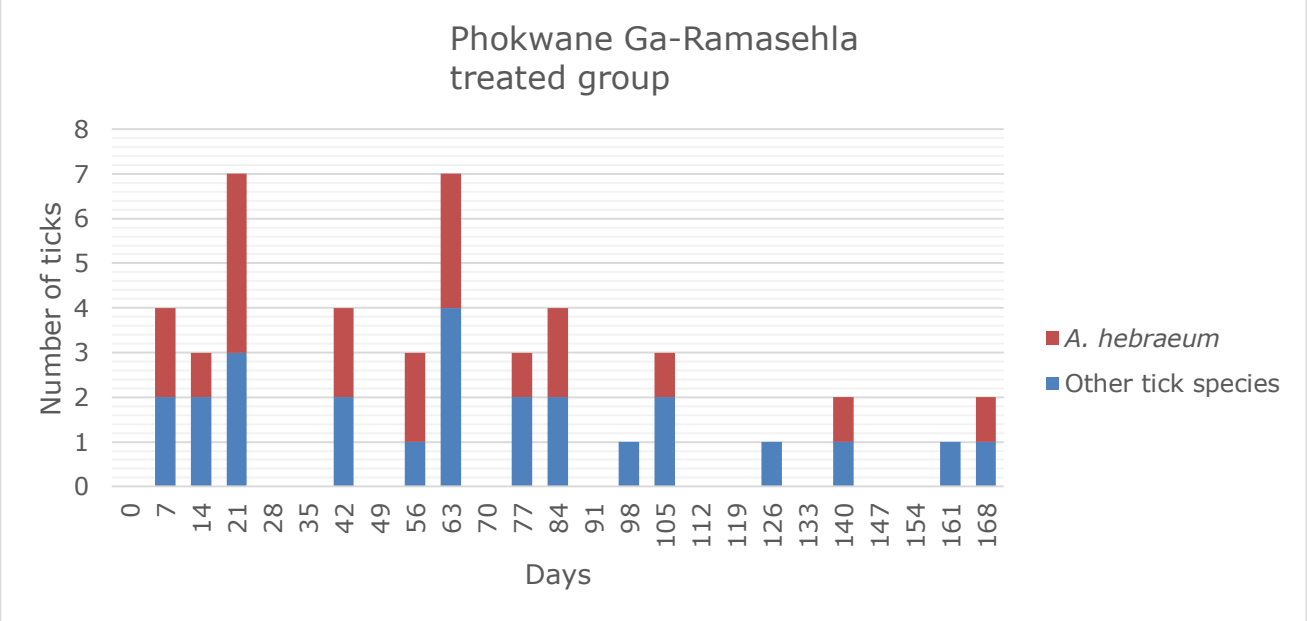

Figure 5b. The total number of *A. hebraeum* and other tick species in a treated group of 5 goats in one household at Phokwane Ga-Ramasehla village.

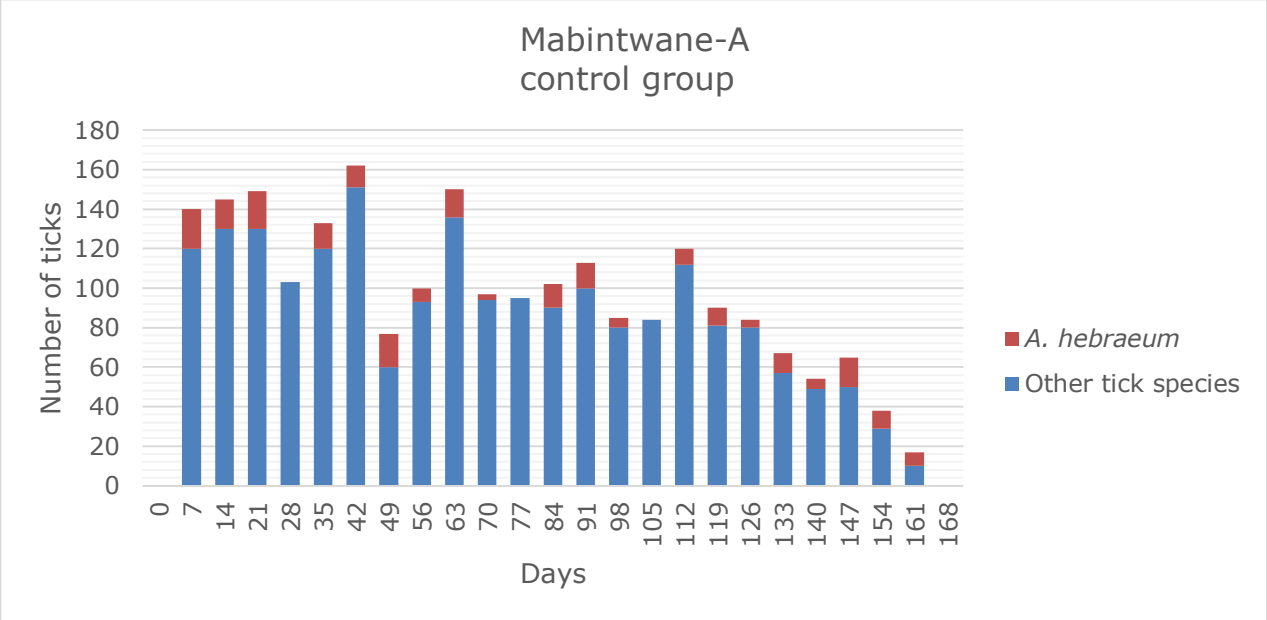

Figure 6a. The total number of *A. hebraeum* and other tick species in a control group of 5 goats at Mabintwane-A village.

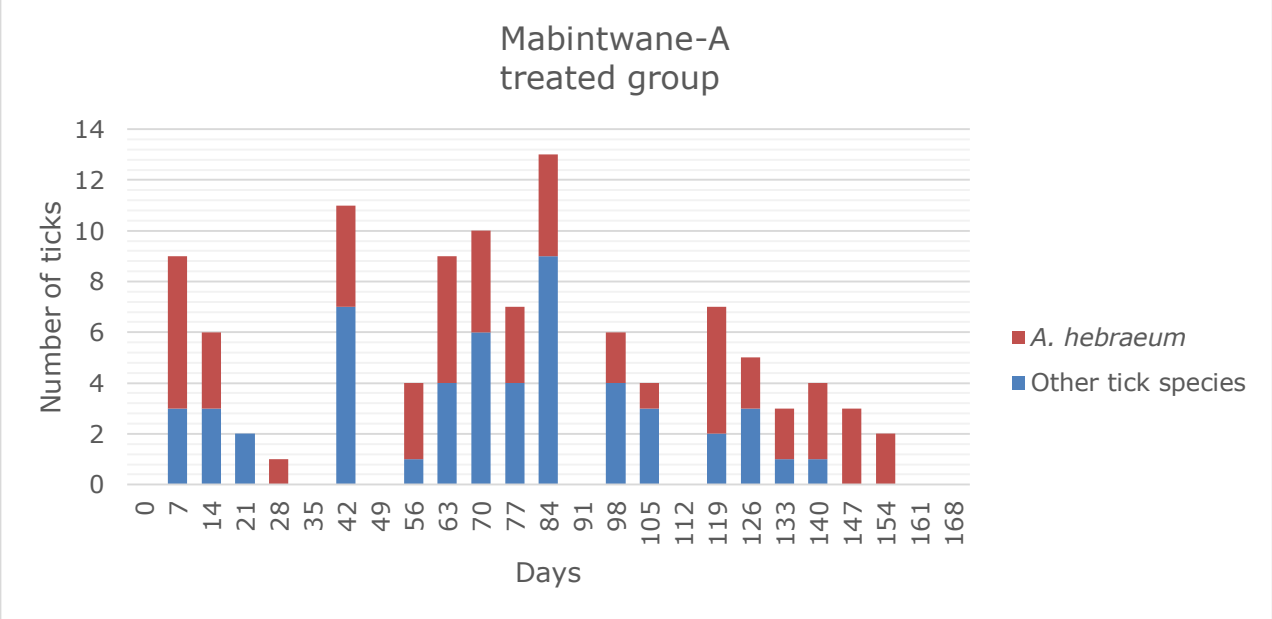

Figure 6b. The total number of *A. hebraeum* and other tick species in a treated group of goats at Mabintwane-A village.

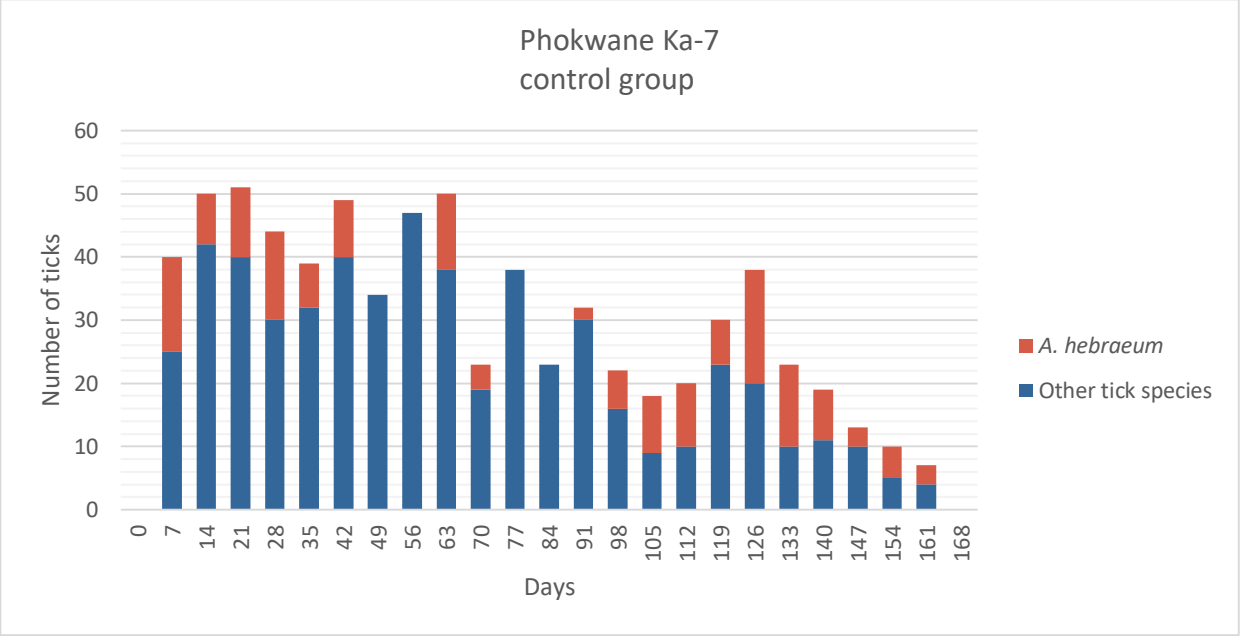

Figure 7a. The total number of *A. hebraeum* and other tick species in a control group of 5 goats in one household at Phokwane village (Ka-7).

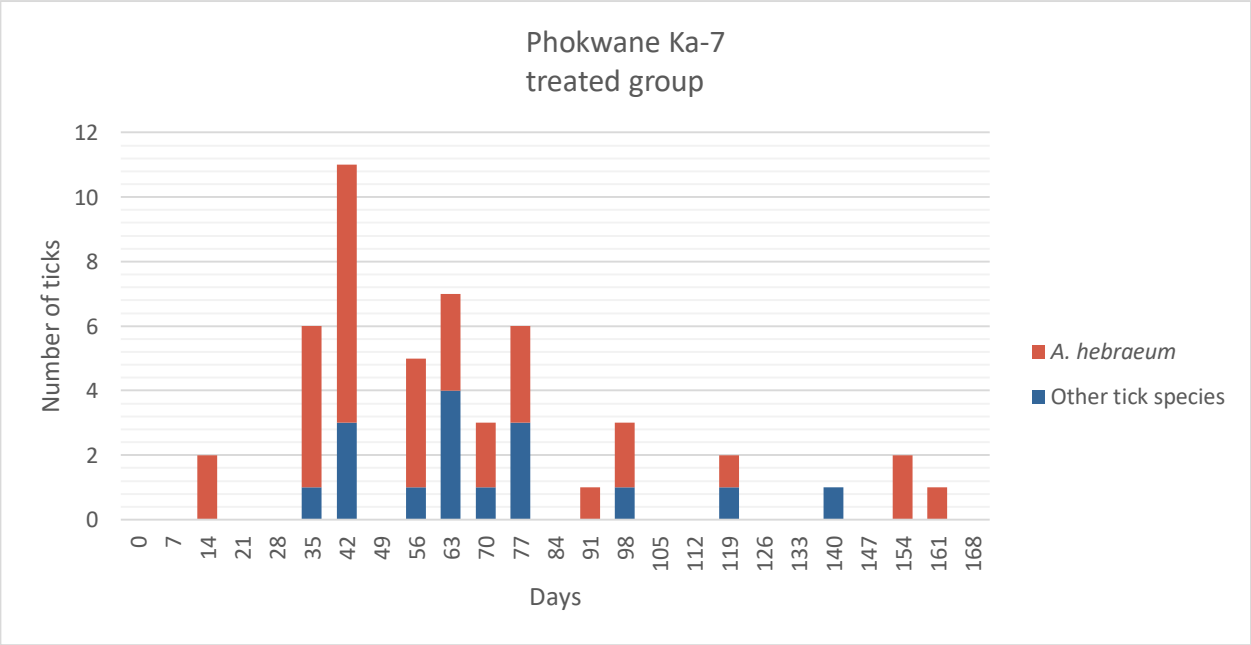

Figure 7b. The total number of *A. hebraeum* and other tick species in a treated group of 5 goats in one household at Phokwane village (Ka-7).

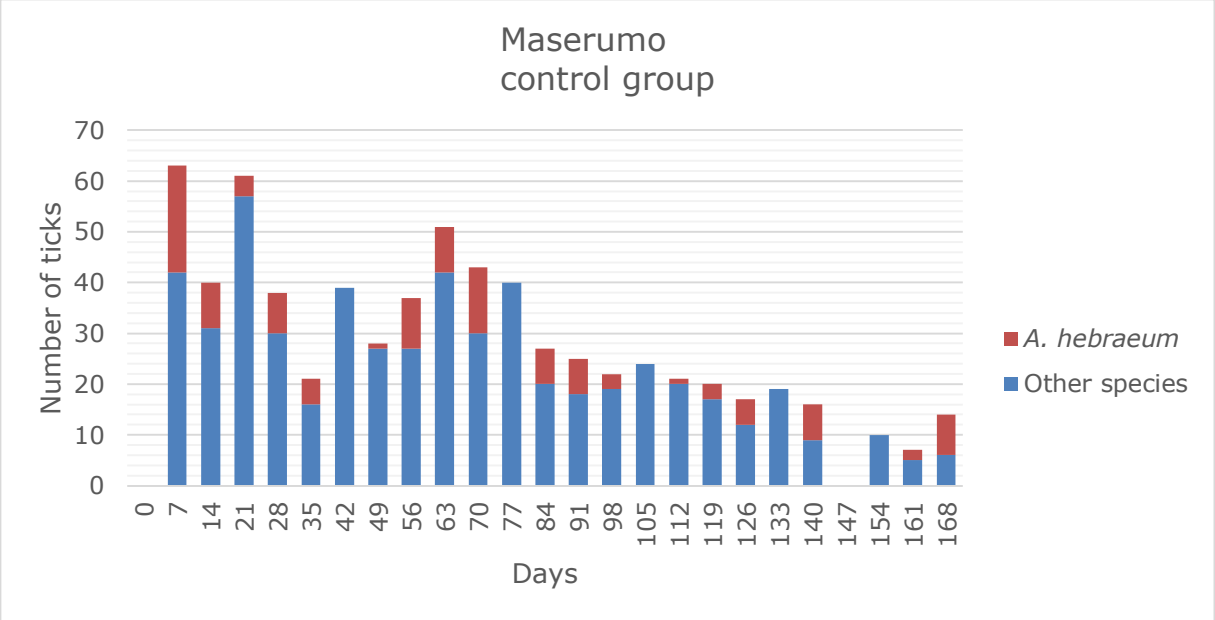

Figure 8a. The total number of *A. hebraeum* and other tick species in a control group of 5 goats in a household at Maserumo village.

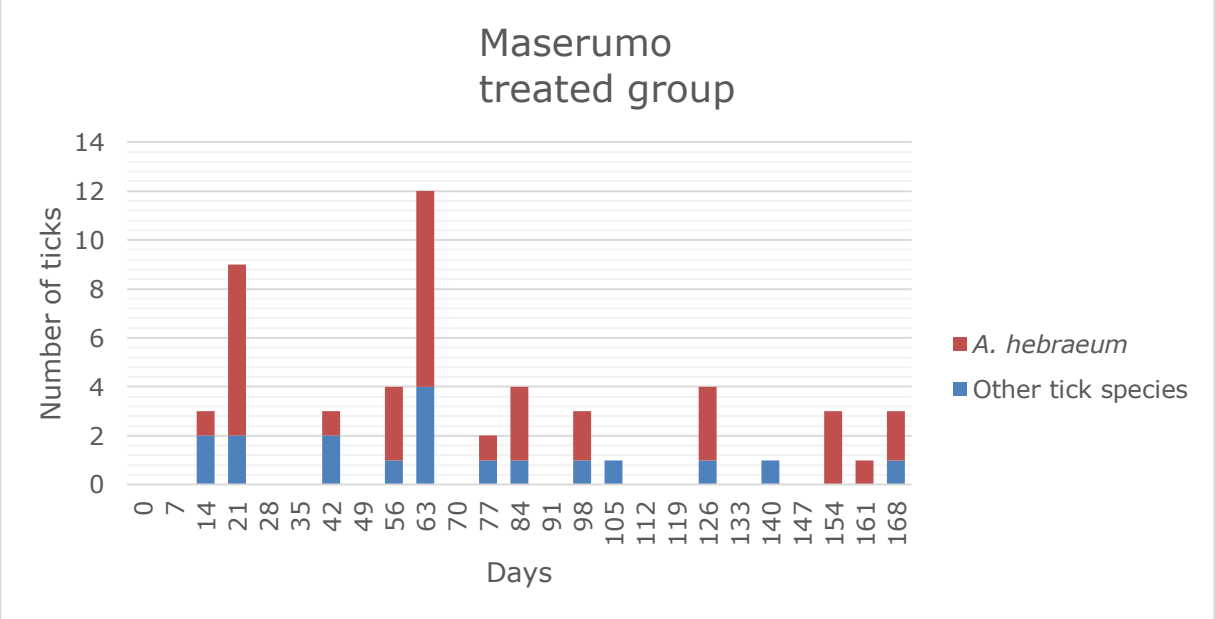

Figure 8b. The total number of A. hebraeum and other tick species in a treated group of 5 goats in a household at Maserumo village.

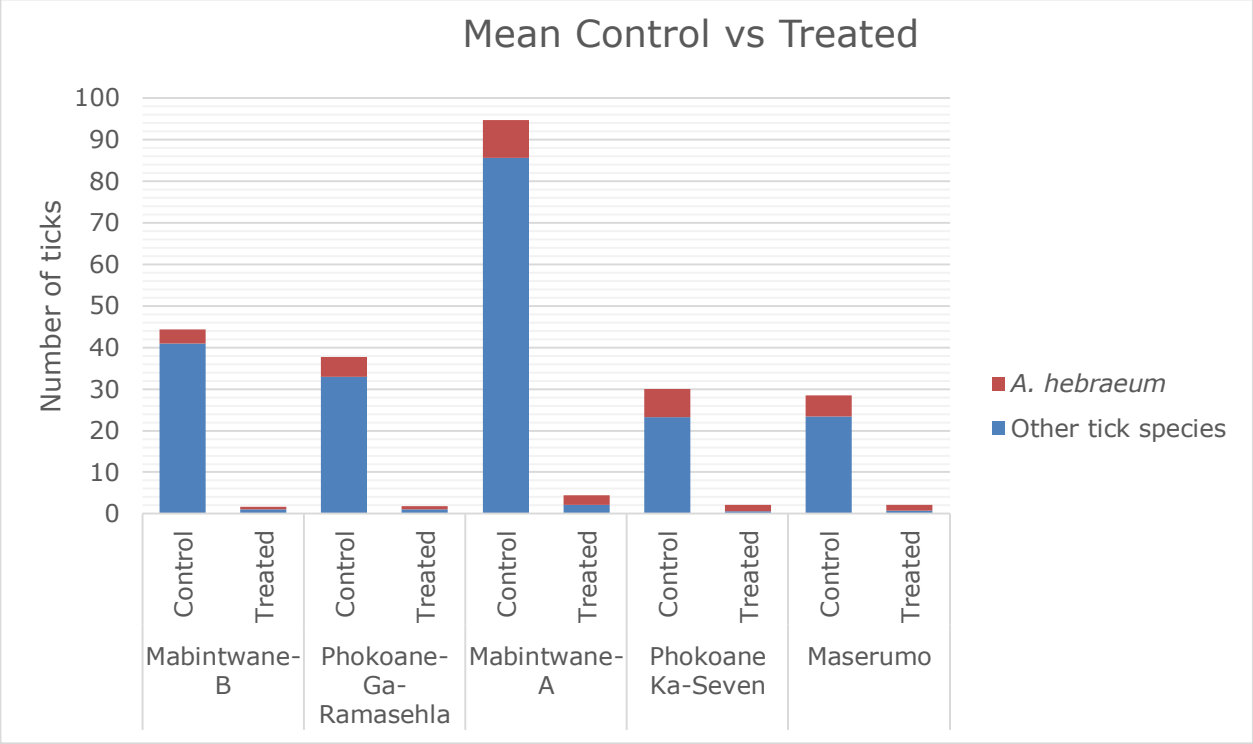

Figure 9. The mean of all ticks counted on treated (25 goats) and control group goats (n=25) from five households in Makhuduthamaga rural area, Sekhukhune district of Limpopo province, South Africa between 15 February and 30 July 2018.

Detection of *E. ruminantium* by pCS20 PCR in *A. hebraeum* GUTS

Additional *Amblyomma* ticks were collected from goats not enrolled in the efficacy trial, but for isolating *E. ruminantium.* These goats were kept by the same owners in three villages of Makhuduthamaga municipality. A total of 400 ticks were collected in batches of 50 ticks on 8 occasions. Most of the ticks were collected from the predilection sides. All ticks were taxonomically identified in the laboratory by microscopy as either adult male, female (not fully engorged) or nymphs of *A. hebraeum* (Table 1). For the subsequent isolation of *E. ruminantium*, GUTS were prepared and stored frozen. One of 8 GUTS was found to be positive for *E. ruminantium* by pCS20 PCR (Figure 10). The gel containing the positive sample is shown in Figure 10. The remaining negative results are not shown. This concerned the tick collection made on 18/06/2018 in the village of Maserumo (Table 2).

Table 1. Additional *Amblyomma* collections from goats not enrolled in the efficacy trial but for isolating *E. ruminantium* kept by same owners in Makhuduthamaga municipality.

Geographical origin	Household	Collected date	Tick species (*A. hebraeum*)	No of ticks collected	Male/Female
Mabintwane-B	1	08-02-18	Adults	47	37 M & 10 F
			Nymphs	3	
Phokwane Ga-Ramasehla	2	02-03-18	Adults	46	30 M & 16 F
			Nymphs	4	
		13-07-18	Adults	47	7 M & 40 F
			Nymphs	3	
Mabintwane-A	3	14-04-18	Adults	43	13 M & 30 F
			Nymphs	7	
		26-05-18	Adults	46	29 M & 17 F
			Nymphs	4	
Phokwane Ka-7	3	06-05-18	Adults	41	34 M & 7 F
			Nymphs	9	
Maserumo	5	26-03-18	Adults	48	38 M & 10 F
			Nymphs	2	
		18-06-18	Adults	46	26 M & 20 F
			Nymphs	4	
No of Adults			**364**		
No of Nymphs			**36**		
No of Guts				**8**	
Total ticks			**400**		

Table 2. Detection of *Ehrlichia ruminantium* in *Amblyomma hebraeum* ticks by PCR.

Geographical location	Household	Date tested	Number of ticks per GUT	Number of ampules	PCR+/–
Mabintwane-B	1	07-08-2018	50	1	–
Phokwane Ga-Ramasehla	2	07-08-2018	50	1	–
Mabintwane-A	3	07-08-2018	50	1	–
Phokwane Ka-7	4	07-08-2018	50	1	–
Maserumo	5	07-08-2018	50	1	–
Phokoane Ga-Ramasehla	2	07-08-2018	50	1	–
Mabintwane-A	3	07-08-2018	50	1	–
Maserumo	5	07-08-2018	50	1	+

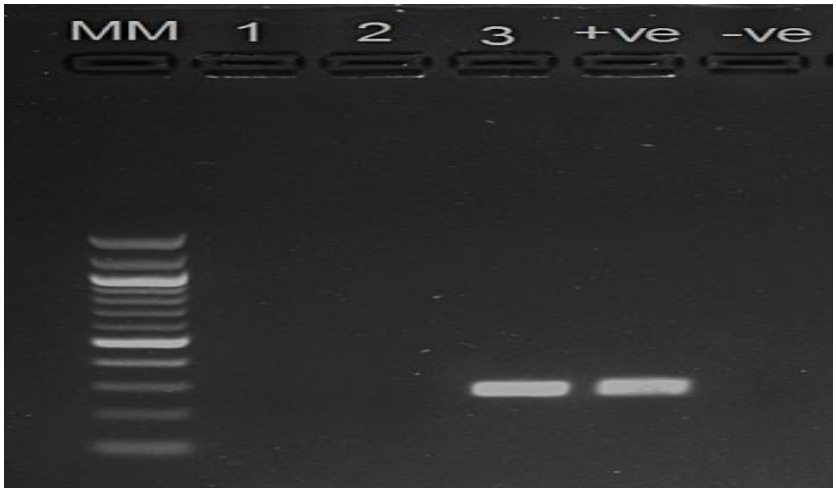

Figure 10. PCR detection of *E. ruminantium* in ground-up *A. hebraeum* tick supernatants

Discussion

Tick control

The aim of this study was to contribute to an improved strategy for the control of tick infestation on indigenous goats owned by emerging farmers in Limpopo Province, South Africa. Firstly, the acaricidal efficacy was determined of a poor-on deltamethrin treatment on goats infested by ticks at Makhuduthamaga rural areas of Nebo in Sekhukhune district (Limpopo province), South Africa. Secondly, *Ehrlichia ruminantium* was detected in field collected *A. hebraeum* ticks and cryopreserved as ground-up-tick supernatants.

Topical treatment with deltamethrin pour-on (Clout®) reduced tick numbers by 94.5% on indigenous goats in the treatment group compared to the control group. In total, 5,671 ticks were collected from the control group with an average of 9.44 ticks per goat, whereas a total of 296 ticks were collected from the treated group with an average of 0.52 ticks per goat. Differences between the number of ticks counted in the treated versus control groups were statistically significant at all times points (Mann-Whitney U test).

Although various irregularities occurred they did not invalidate the study. It underlines the limitations when working with animals owned by farmers, who are not always able to follow a study protocol. Although the general conclusions of the study remain unchanged, it is difficult to determine precisely the impact of the goat replacement during the study.

The relative proportion of *Amblyomma* ticks was 56% in the treatment group versus 12% in the control group. The efficacy of the topical treatment with deltamethrin pour-on (Clout®) for control of *Amblyomma* ticks only was 72%. It appears that *Amblyomma* ticks may be more difficult to control and that these ticks re-infest goats more successfully than other tick species. However, male *A. hebraeum* remain attached to their hosts, even after treatment. This may explain why the cumulative number of *Amblyomma* ticks is higher on treated goats than on the controls.

Although it is important to gain insight in the overall species composition and distribution of ticks infesting goats, ticks other than *Amblyomma* were only counted and not identified in this study. Ticks found on goats may play a role in the transmission of diseases other than heartwater, not necessarily only for goats, but also for other livestock (NYANGWIWE & HORAK, 2007). The role of goats in the epidemiology of cattle ticks has been emphasised by various authors (BAKER & DUCASSE, 1968; DE MATOS et al. 2009).

A previous study conducted in Mpumalanga and in the North West Province between 1991 and 1993, wherein ticks were collected from indigenous goats owned by small-scale farmers, revealed that *A. hebraeum* was the most common tick, followed by *R. appendiculatus* and *R. evertsii* (BRYSON et al. 2002). This was recently confirmed in the Mnisi Community Area of Mpumalanga Province, where *A. hebraeum* was the predominant tick species collected from goats followed by *Rhipicephalus microplus*. Small numbers of *R. appendiculatus, R. simus* and *R. zambeziensis* adults were also identified (JONGEJAN et al., submitted for publication).

A slightly different situation with respect to species composition on domestic goats was reported from Zimbabwe, where *R. evertsii* was the predominant species (HOVE et al., 2008). From neighbouring Maputo province in Mozambique, a similar species composition was reported with *A. hebraeum, R. appendiculatus, R. microplus, R. evertsii and R. simus* (DE MATOS et al., 2009).

Furthermore, it was observed that the number of ticks counted on the control goats slowly declined during the trial, which coincided with the approach of winter, wherein conditions for ticks are less favourable conditions for ticks. The seasonal abundance of ticks associated with indigenous goats has been previously investigated in the northern Transvaal (RECHAV and DE JAGER, 1991).

Lameness of goats was often observed by the owners interviewed in this study. Furthermore, they observed massive infestation of ticks on the feet of their goats and observed lameness, in particular during the summer. The association between foot abscesses in goats and the seasonal occurrence of adult *A. hebraeum*, but also of adult *Rhipicephalus glabroscutatum* ticks, has been reported previously (MACIVOR and HORAK, 1987). Another tick that is

notorious with respect to foot infestations and temporary lameness is *Hyalomma rufipes*, in particular on Merino sheep in the Free State of South Africa (KOK and FOURIE, 1995). Although the benefit of the deltamethrin treatment with respect to indirectly reducing the damage due to ticks (lameness) could not be fully evaluated in this limited number of goats; the intervention is expected to lead to improved animal health and wellbeing,

The household goat owners that participated in this study were using traditional remedies to perform tick control. However, these remedies did not appear effective at all considering the massive tick infestations found on day zero when examining the goats. On the other hand, pour-on Clout® was used in this study since it contains 1% deltamethrin and the product is registered for treatment of cattle, sheep and goats (See Appendix F).

A visit to Groblersdal to purchase acaricides is worth the effort. If an individual household needs to treat their goats once per month, it will cost R14.18 monthly utilising a 5 L bottle (based on an average number of 6.5 goats per farmer). However, deltamethrin treatment was found to lose its efficacy towards day 21. The results of this study in the treated group of goats clearly showed that the number of ticks increased against to the previous level before the next acaricidal application. Therefore, the treatment of goats every fortnight may be required, in particular during the summer months.

Finally, the results of the questionnaire surveys clearly demonstrated that the household goat owners were highly appreciative of the innovative strategy of tick control and aroused the interest of other owners not directly involved in this field trial.

Ehrlichia ruminantium infection

Since deltamethrin dramatically reduced the tick burden on treated goats, these animals may have a lower risk of succumbing to heartwater in comparison with the non-treated control goats, since the challenge of infected ticks is much lower. One household goat owner stipulated that some of his goats had died after walking in a circle, paddling with their legs and with a protrusion of the neck. This is most likely due to *E. ruminantium* infection in the brain, which is typical for clinical heartwater cases. Although this requires confirmation by the

detection of rickettsial inclusion bodies in endothelial cells of capillaries in the brain, it suggests that indigenous breeds of goats are more susceptible to heartwater than assumed.

These observations underpin the importance of isolating current strains of heartwater to further our understanding of the disease dynamics and pathogenicity for small ruminants. Therefore, in addition to the efficacy trial, additional *Amblyomma* ticks were collected from goats not enrolled in the efficacy study, but kept by the same households and processed for the isolation and preservation of possible novel heartwater strains from the field to facilitate further studies. One of the 8 GUTS (12.5%) was found to be positive for *E. ruminantium* by pCS20 PCR. This is much lower than the positivity of 8 out of 8 GUTS tested positive in a previous study conducted by NEL (2018). Finally, these ground-up-tick-supernatants need to be tested for their infectivity to induce heartwater in experimental goats and adapted to *in vitro* growth in endothelial cell cultures. This will broaden the array of currently available heartwater strains and facilitate our understanding of the disease dynamics, ultimately leading to improved disease control.

Conclusion and recommendation

The hypothesis that topical treatment with a deltamethrin pour-on solution will reduce tick numbers by 90% on indigenous goats in the treatment group compared to the control group could be accepted since the efficacy found in this study was 94.5%. From the data collected during this study, it was concluded that the pour-on deltamethrin was effective in preventing tick infestation on goats. However, the acaricidal application interval may have to be reduced to two weeks, since ticks were already re-infesting the goats toward the end of the application interval of three weeks. It is recommended to include goats into strategic tick control programmes in order to reduce lameness due to *Amblyomma* tick infestation and the risk of contracting heartwater disease. In this study, *E. ruminantium* was detected by PCR in field collected *A. hebraeum* ticks and cryopreserved as a ground-up-tick supernatant to facilitate further studies on heartwater. The isolation of current heartwater strains is an important step with respect to conducting research on this important tick-borne disease.

Finally, continuing to inform farmers about sustainable and affordable tick control methods is recommended, as this will lead to measurable improvements of livelihoods and the adoption by other farmers of the study model once the benefits have been clearly demonstrated.

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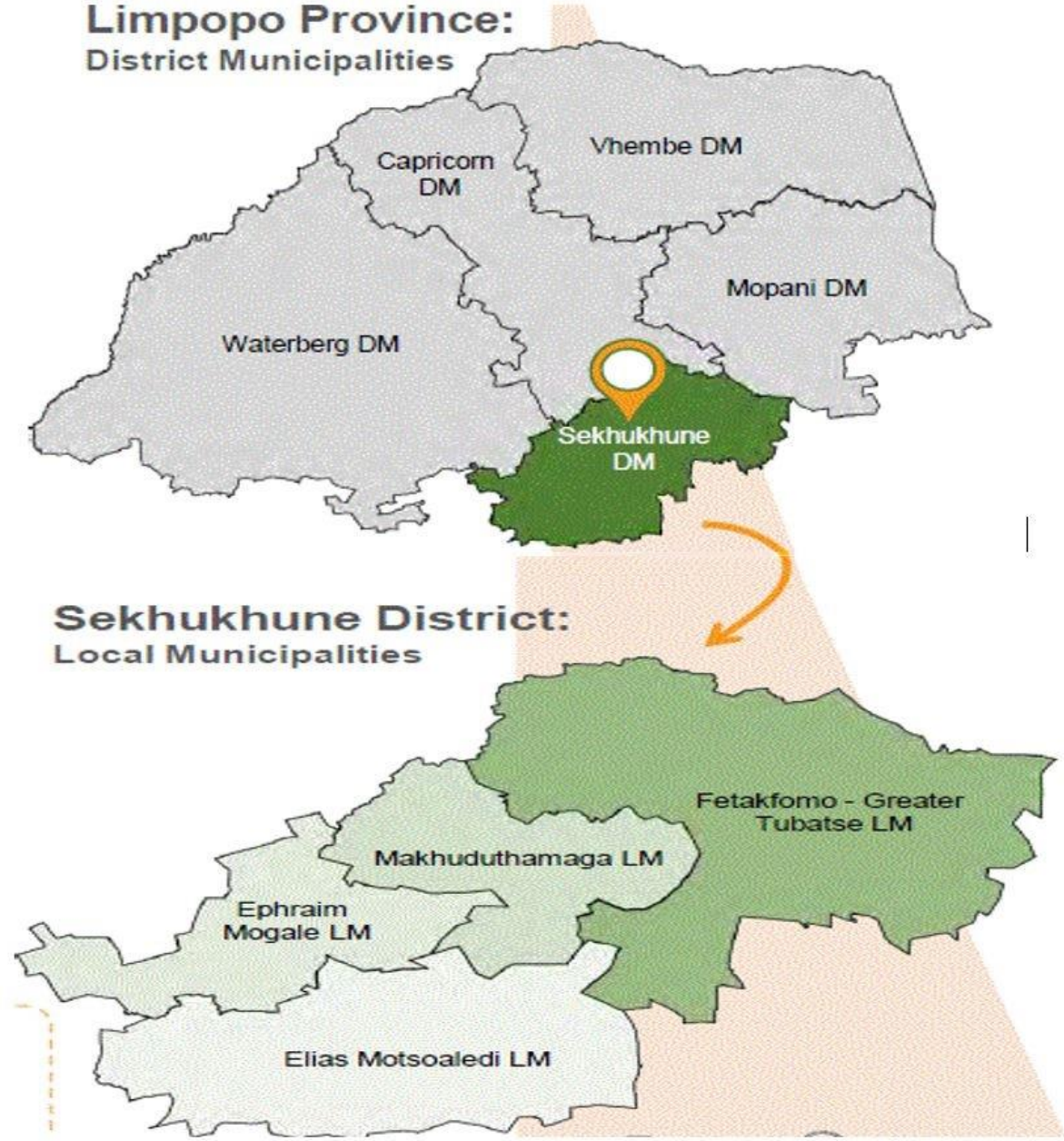
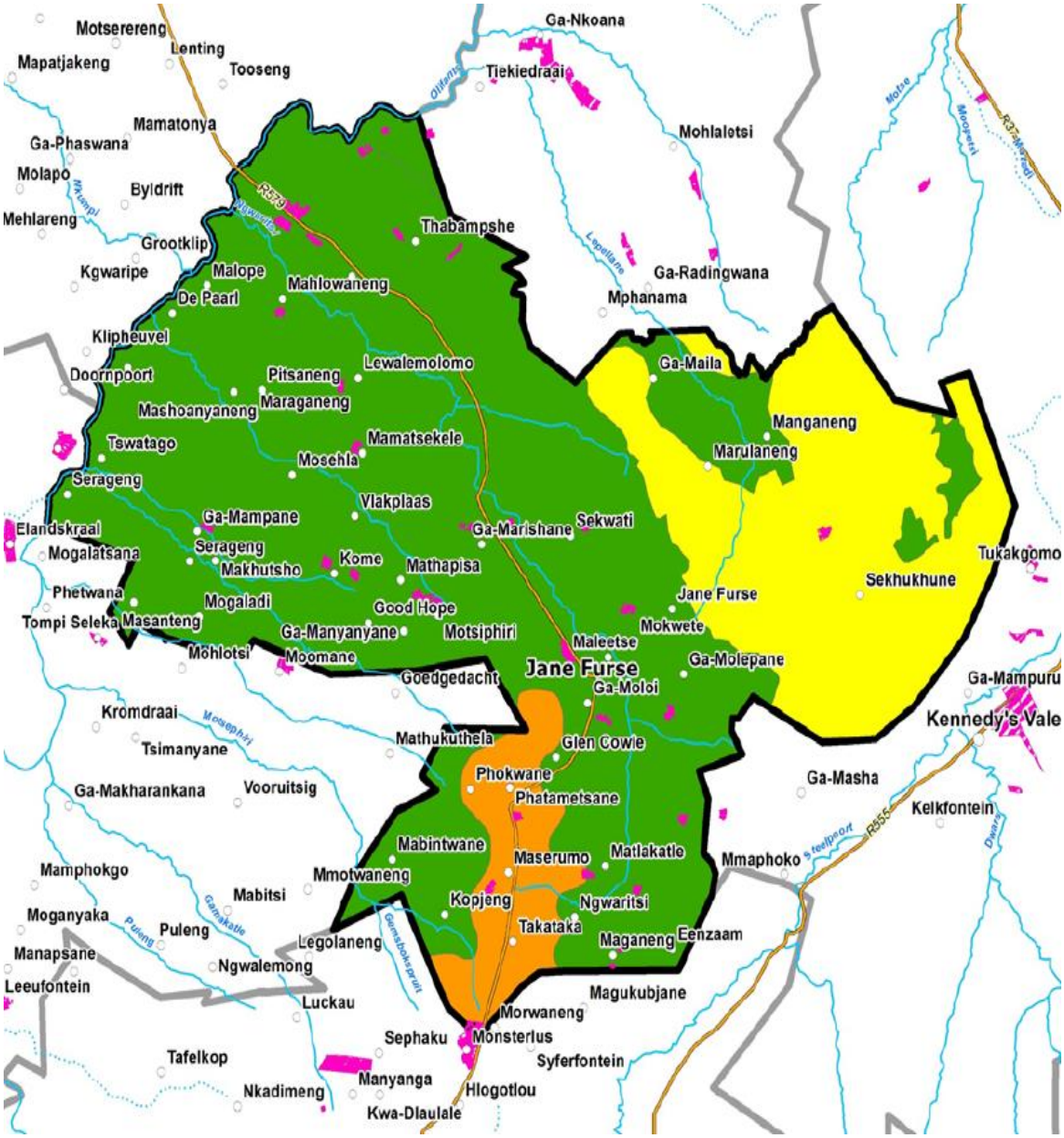
Appendix A: Study area

Map of Limpopo province showing 5 districts municipalities and Sekhukhune district with 4 local municipalities where the study conducted in Makhuduthamaga local municipality's rural areas of Nebo.



Map of Makhuduthamaga local municipality (MLM) showing their rural areas (Phokwane, Mabintwane and Maserumo located at the bottom corner).

Mabintwane-B (Latitude: 24° 54’ 58” **S** and Longitude: 29° 39’ 32” **W**); **Phokwane Ga-Ramasehla** (Latitude: 24° 51’ 2” **S** and Longitude 29° 42’ 15” **W**); **Mabintwane-A** (Latitude: 24° 54’27” **S** and Longitude: 29° 40’ 58” **W**); **Phokwane Ka-7** (Latitude: 24° 51’ 5” **S** and Longitude: 29° 43’ 57” **W**); **Maserumo** (Latitude: 24° 54’ 50” **S** and Longitude: 29° 45’ 32” **W**)



Maps by Makhuduthamaga local municipality (2014/2015 and 2018) edited by Mashifane M.K. (2019).

Appendix B: Approval from the Animal Ethics Committee (AEC) of the University of Pretoria (Project number V119-17)

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Animal Ethics Committee

PROJECT TITLE	**Acaricidal efficacy of CLOUR® against *Amblyomma* ticks on goats in Limpopo province (Sekhukhune District) and isolation of *Ehrlichia ruminantium* strains**
PROJECT NUMBER	**V119-17**
RESEARCHER/PRINCIPAL INVESTIGATOR	**MM Kgoogole**

STUDENT NUMBER (where applicable)	**U_10216147**
DISSERTATION/THESIS SUBMITTED FOR	**MSc**

ANIMAL SPECIES	**Goats**	**Ticks**
NUMBER OF ANIMALS	**50**	**400**
Approval period to use animals for research/testing purposes		**November 2017- November 2018**
SUPERVISOR	**Prof. F Jongejan**	

KINDLY NOTE:

Should there be a change in the species or number of animal/s required, or the experimental procedure/s - please submit an amendment form to the UP Animal Ethics Committee for approval before commencing with the experiment

APPROVED	Date 27 November 2017
CHAIRMAN: UP Animal Ethics Committee	Signature

Appendix C. Permission to conduct research in terms of section 20 of the Animal Diseases Act (Act 35 of 1984) from the Department of Agriculture Forestry and Fisheries (DAFF).

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Henry Goldie • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HenryG@daff.gov.za
Reference: 12/11/1/1/6

Mashifane Marnaje Kgaogelo
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: +31 030 253 2568
Email: F.Jongejan@uu.nl; kmamaje@yahoo.com

RE: PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984)

Dear Mr Kgaogelo

Your application dated 8 November 2017 requesting permission under Section 20 of the Animal Disease Act, 1984 (Act No. 35 of 1984) to perform a research project or study, refers. I am pleased to inform you that permission is hereby granted to perform the following study, with the following conditions:

Conditions:

1. This permission does not relieve the researcher of any responsibility which may be placed on him by any other act of the Republic of South Africa;
2. The study is approved as per the application form dated 8 November 2017 and the correspondence thereafter. Written permission from the Director: Animal Health must be obtained prior to any deviation from the conditions approved for this study under this Section 20 permit. Please apply in writing to HenryG@daff.gov.za;
3. If required, an application for an extension must be made by the responsible researcher at least one month prior to the expiry of this Section 20 permit. Please apply in writing to HenryG@daff.gov.za;
4. No part of this study may begin until the valid ethical approval has been obtained in writing from the relevant South African authority;

– 1 –

5. Written consent from the owners of the animals to be used in this study must be obtained by the researcher prior to commencement of the study;
6. Only products registered in terms of the Fertilizers, Farm Feeds, Agricultural Remedies and Stock Remedies Act 1947 (Act no 36 of 47) may be used in this project and all relevant withdrawal periods must be adhered to prior to any slaughter of these animals;
7. All ticks must be securely packaged in a manner to prevent their escape during transportation and in compliance with the National Road Traffic Act 1996 (Act no 93 of 96);
8. All collected ticks must be destroyed at completion of the study and must not be used for any purpose other than to obtain "Ground-up-Tick-Supernatant" directly from them;
9. All potentially infectious material utilised or generated during or by the study is to be destroyed at completion of the study;
10. Only a registered waste disposal company may be used for the removal of all potentially infectious waste from the study;
11. Records must be kept for five years for auditing purposes;
12. A dispensation for the storage of Ground-up-Tick-Supernatant from collected ticks is attached.

Title of research/study: Acaricidal efficacy of CLOUT® against *Amblyomma* ticks on goats in Limpopo Province (Sekhukhune District) and isolation of *Ehrlichia ruminantium* strains.
Researcher: Mashifane Mamaje Kgaogelo
Institution: Department of Veterinary Tropical Diseases; University of Pretoria.
Permit Expiry date: 30 June 2018
Our ref Number: 12/11/1/1/6 (631)
Your ref: N/A

Kind regards,

DR. MPHO MAJA
DIRECTOR OF ANIMAL HEALTH
Date: 2017 -12- 05

SUBJECT: *PERMISSION TO DO RESEARCH IN TERMS OF SECTION 20 OF THE ANIMAL DISEASES ACT, 1984 (ACT NO. 35 OF 1984)*

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Animal Health, Department of Agriculture, Forestry and Fisheries
Private Bag X138, Pretoria 0001

Enquiries: Mr Herry Gololo • Tel: +27 12 319 7532 • Fax: +27 12 319 7470 • E-mail: HerryG@daff.gov.za
Reference: 12/11/1/1/6

Mashifane Mamaje Kgaogelo
Department of Veterinary Tropical Diseases
University of Pretoria
Tel: +31 030 253 2568
Email: F.Jongejan@uu.nl; kmamaje@yahoo.com

RE: DISPENSATION ON SECTION 20 APPROVAL IN TERMS OF THE ANIMAL DISEASES ACT, 1984 (ACT NO 35 OF 1984) FOR: "ACARICIDAL EFFICACY OF CLOUT® AGAINST *AMBLYOMMA* TICKS ON GOATS IN LIMPOPO PROVINCE (SEKHUKHUNE DISTRICT) AND ISOLATION OF *EHRLICHIA RUMINANTIUM* STRAINS"

A dispensation is hereby granted on Point 9 of the Section 20 approval that was issued for the above mentioned study (attached):

i) Ground-up-Tick-Supernatant from collected ticks must be stored under access control at the Department of Veterinary Tropical Diseases at the Faculty of Veterinary Sciences, University of Pretoria;

ii) Ground-up-Tick-Supernatant from collected ticks must not be outsourced or used for further research without prior written approval from the Director: Animal Health.

Kind regards,

DR. MPHO MAJA
DIRECTOR: ANIMAL HEALTH
Date: 2017 -12- 05

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF AGRICULTURE

MAKHUDUTHAMAGA MUNICIPALITY

Enquiries: Dr. Sekokotla M J – State Vet Makhuduthamaga

Date: 06 November 2017

Subject: Letter of Permission for Mr. Kgaogelo Mashifane to do a research study with Makhuduthamaga Farmers

To Whom It May Concern

I hereby state that State Vet Makhuduthamaga office support Mr. Kgaogelo Mashifane to do a research study with Makhuduthamaga Small Stock Farmers in line with our Limpopo Department of Agriculture Director for Veterinary Services letter dated 03 October 2017 permitting him to do a research study at Makhuduthamaga with Small Stock Farmers.

Yours

SIGNED

Dr. Sekokotla 079 707 1267

- **BVMCh 1990 MEDUNSA**
- **Magister Scientiae M. Sc (Vet Sciences) 2004 Pretoria University**

Private Bag X125, Nebo, 1059

Tel: (013) 264 0057/0484/5 Fax: (013) 264 8213 Website: http://www.lda.gov.za

The heartland of Southern Africa - development is about people!

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
AGRICULTURE AND RURAL DEVELOPMENT

Mashifane M.K
P.O. Box 841
NEBO
1059

Director Veterinary Services
Limpopo Department of Agriculture and Rural Development
Private Bag X9847
POLOKWANE
0700
03 November 2017

Dear Mr Mashifane

REQUEST FOR PERMISSION UNDER SECTION 20 OF THE ANIMAL DISEASES ACT (ACT 35 OF 1984) TO PERFORM RESEARCH / STUDY

I, Dr R.L. Mampane (Director Veterinary Services Limpopo Province), hereby declare that I take note and have no objection for the following study to be undertaken in the Sekhukhune District of Limpopo Province;

"Control of Amblyoma hebraeum tick using CLOUT on indigenous goats of emerging farmers in Nebo rural areas, Limpopo Province, South Africa"

The area is not under any disease restriction.

As the study will involve collection of ticks under controlled conditions in the Foot and Mouth Disease-Free area of the Province with no disease risk, we have no objection to the exercise.

The Research is supported.

Yours sincerely,

Dr. R. L. Mampane
Director of Veterinary Services
LIMPOPO PROVINCE

Appendix D: Questionnaires

DATA CAPTURE:	QUESTIONNAIRE-TICK COLLECTION FROM GOATS FORM
Sampling date	08-February-2018
Village name	Mabintwane-B
GPS household	24° 54’ 58” S and 29° 39’ 32” W
Name of owner	Nyathi T.
Total number of goats	12
Number of sampled goats	5
Number of ticks collected	50
Tick tube(s) ID	1

Does the owner perform any tick control?	Yes.
What kind of tick control is performed?	Spraying unknown.
How often does the owner perform tick control?	Once a week.
How many goats (suddenly) died last year?	1 & 2 due cannibalism.
Has the owner seen pedalling movements just before goat(s) died?	Yes, on 3 goats.
Has the owner seen lameness/infected feet?	Yes, mostly in summer.
How is the goat(s) treated and how often?	No treatment.
Would the owner be interested in new tick control methods?	Yes, highly interested.

Comment(s)

Their strategy of tick control with an unknown remedy they used seems not effective since more ticks found on day zero when clout® applied to treated group.

Form filled by: Mashifane M.K. **Date:** 08 February 2018

DATA CAPTURE: FORM	QUESTIONNAIRE-TICK COLLECTION FROM GOATS
Sampling date	02-March-2018
Village name	Phokwane Ga-Ramasehla
GPS household	24° 51’ 2” S and 29° 42’ 15” W
Name of owner	Masehla B.
Total number of goats	39
Number of sampled goats	5
Number of ticks collected	50
Tick tube(s) ID	2

Does the owner perform any tick control?	Yes.
What kind of tick control is performed?	Unknown spray.
How often does the owner perform tick control?	Once a month.
How many goats (suddenly) died last year?	8 kids.
Has the owner seen any pedalling movements just before goat(s) died?	No, sudden death
Has the owner seen lameness/infected feet?	Yes, on kids.
How is the goat(s) treated and how often?	Wound spray.
Would the owner be interested in new tick control methods?	Yes.

Comment(s)

It seems like the owner does not regularly observe his flock if for instance such a large number of kids can die without noticing the cause. I therefore suspect heartwater since most of them are kids. Most of the goats went missing when returning from the veld.

Form filled by: Mashifane M.K. **Date:** 02 March 2018

DATA CAPTURE: FORM	QUESTIONNAIRE-TICK COLLECTION FROM GOATS
Sampling date	14-April-2018
Village name	Mabintwane-A
GPS household	24° 54’ 27” S and 29° 40’ 58” W
Name of owner	Boshielo J.
Total number of goats	45
Number of sampled goats	5
Number of ticks collected	50
Tick tube(s) ID	3

Does the owner perform any tick control?	No.
What kind of tick control is performed?	X
How often does the owner perform tick control?	X
How many goats (suddenly) died last year?	9 kids, 2 adults.
Has the owner seen pedalling movements just before goat(s) died?	Yes, to 7 kids.
Has the owner seen lameness/infected feet?	Yes, rainy season.
How is the goat(s) treated and how often?	No treatment.
Would the owner be interested in new tick control methods?	Yes, much interest.

Comment(s)

X= means no comment. In this household, the owner wanted the study to include the entire flock in terms of tick control. Stock theft seems very stressful for the owner.

Form filled by: Mashifane M.K. **Date:** 14 April 2018

DATA CAPTURE: FORM	QUESTIONNAIRE-TICK COLLECTION FROM GOATS
Sampling date	06-May-2018
Village name	Phokwane Ka-7
GPS household	24° 51’ 5” S and 29° 43’ 57” W
Name of owner	Mmakola T.
Total number of goats	95
Number of sampled goats	5
Number of ticks collected	50
Tick tube(s) ID	4

Does the owner perform any tick control?	No.
What kind of tick control is performed?	X
How often does the owner perform tick control?	X
How many goats (suddenly) died last year?	10 kids, 6 adults.
Has the owner seen pedalling movements just before goat(s) died?	Yes, in autumn.
Has the owner seen lameness/infected feet?	Yes, in summer
How is the goat(s) treated and how often?	Wound spray.
Would the owner be interested in new tick control methods?	Yes.

Comment(s)

X= means no comments. The owner emphasised that he is very interested in new tick control strategies. However, he has insufficient funds to purchase them and the stores selling acaricide are sparse. Most of his goats were lost due to stock theft and cannibalism.

Form filled by: Mashifane M.K. **Date:** 06 May 2018

DATA: QUESTIONNAIRE-TICK COLLECTION FROM GOATS FORM	
Sampling date	26-March-2018
Village name	Maserumo
GPS household	24° 54’ 50” S and 29° 45’ 32” W
Name of owner	Sekgala M.
Total number of goats	16
Number of sampled goats	5
Number of ticks collected	50
Tick tube(s) ID	5

Does the owner perform any tick control?	Yes.
What kind of tick control is performed?	Spray with paraffin.
How often does the owner perform tick control?	Once in 2 months.
How many goats (suddenly) died last year?	3 kids & 2 adults.
Has the owner seen pedalling movements just before goat(s) died?	Yes, 1 kid & 2 adults.
Has the owner seen lameness/infected feet?	Yes, 2 kids & 1 adult
How is the goat(s) treated and how often?	Paraffin on hooves.
Would the owner be interested in new tick control methods?	Yes, very interested.

Comment(s)

Most of the kids in the summer and autumn seem to show signs of lameness. Since the number of goats was less, the possibility of collecting 50 ticks was also lower; therefore, additional *A. hebraeum* were collected from other household goats of the same village.

Form filled by: Mashifane M.K. **Date:** 26 March 2018

Appendix E: Selected tick counting sheets

DATA CAPTURE:	TICK COUNTING SHEET	DAY: 7

Sampling date	15-February-2018
Village name	Mabintwane-B
GPS household	24° 54’ 58”S and 29° 39’ 32” W
Name of owner	Nyathi T.
Total number of goats	12
Number of sampled goats	5
Number of ticks counted	89
Tick tube(s) ID	1

Treated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	0	0	0	0	0	
Armpits	0	0	0	0	0	
Back legs	0	0	0	0	0	
Udder/scrotum	0	0	0	0	0	
Ears	0	0	0	0	0	
Tail	0	0	0	0	0	
Body	0	0	0	0	0	
Total ticks	**0**	**0**	**0**	**0**	**0**	**0**

Control untreated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	5	0	3	3	2	
Armpits	0	3	7	0	6	
Back legs	1	6	2	4	0	
Udder/scrotum	2	2	3	1	0	
Ears	4	3	0	0	1	
Tail	0	0	0	8	9	
Body	3	4	1	2	4	
Total ticks	**15**	**18**	**16**	**18**	**22**	**89**

Comment(s)

The maximum of 81 other ticks and the minimum of 8 *A. hebraeum* counted in the untreated group

Form filled by: Mashifane M.K. **Date:** 15 February 2018

DATA CAPTURE:	TICKCOUNTING SHEET	DAY: 14

Sampling date	23-February-2018
Village name	Phokwane Ga-Ramasehla
GPS household	24° 51' 2" S and 29° 42' 15" W
Owner name	Masehla B.
Total number of goats	39
Number of sampled goats	5
Number of ticks counted	53
Tick tube(s) ID	2

Treated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	0	0	0	0	0	
Armpits	0	0	0	0	0	
Back legs	0	0	1	0	0	
Udder/scrotum	0	0	0	0	1	
Ears	0	0	0	0	0	
Tail	0	1	0	0	0	
Body	0	0	0	0	0	
Total ticks	**0**	**1**	**1**	**0**	**1**	**3**

Control untreated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	5	0	3	2	2	
Armpits	2	1	2	3	0	
Back legs	0	1	0	0	1	
Udder/scrotum	2	3	0	5	0	
Ears	1	1	3	0	2	
Tail	3	0	1	2	1	
Body	1	2	0	0	1	
Total ticks	**14**	**8**	**9**	**12**	**7**	**50**

Comment(s)

Only one *A. hebraeum* was found in the treatment group

Form filled by: Mashifane M.K. **Date:** 23 February 2018

DATA CAPTURE:	TICK COUNTING SHEET	DAY: 21

Sampling date	03-March-2018
Village name	Mabintwane-A
GPS household	24° 54’ 27” S and 29° 40’ 58” W
Name of owner	Boshielo J.
Total number of goats	45
Number of sampled goats	5
Number of ticks counted	51
Tick tube(s) ID	3

Treated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	1	0	0	0	0	
Armpits	0	0	0	0	0	
Back legs	0	1	0	0	0	
Udder/scrotum	0	0	0	0	0	
Ears	0	0	0	0	0	
Tail	0	0	0	0	0	
Body	0	0	0	0	0	
Total ticks	**1**	**1**	**0**	**0**	**0**	**2**

Control untreated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	3	7	0	1	8	
Armpits	10	0	3	5	3	
Back legs	5	0	9	2	5	
Udder/scrotum	1	6	3	8	1	
Ears	0	9	0	4	13	
Tail	6	3	7	10	0	
Body	5	1	2	6	3	
Total ticks	**30**	**26**	**24**	**36**	**33**	**149**

Comment(s)

Larger number of ticks found in the control group compared to the treatment; therefore, acaricide Clout® seems effective

Form filled by: Mashifane M.K. **Date:** 03 March 2018

DATA CAPTURE:	TICK COUNTING SHEET	DAY: 77

Sampling date	29-April-2018
Village name	Phokwane Ka-7
GPS household	24° 51' 5" S and 29° 43' 57" W
Name of owner	Mmakola T.
Total number of goats	95
Number of sampled goats	5
Number of ticks counted	44
Tick tube(s) ID	4

Treated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	0	0	0	0	0	
Armpits	1	0	0	0	0	
Back legs	0	0	0	0	0	
Udder/scrotum	0	2	0	0	0	
Ears	0	0	1	0	0	
Tail	0	0	0	1	0	
Body	0	0	0	0	1	
Total ticks	**1**	**2**	**1**	**1**	**1**	**6**

Control untreated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	1	0	3	2	2	
Armpits	2	1	4	1	0	
Back legs	0	2	0	3	3	
Udder/scrotum	3	0	0	0	0	
Ears	0	3	0	1	0	
Tail	0	0	2	0	0	
Body	2	0	1	0	2	
Total ticks	**8**	**6**	**10**	**7**	**7**	**38**

Comment(s)

Acaricide Clout® seems to lose its value from 21 days after application.

Form filled by: Mashifane M.K. **Date:** 29 April 2018

DATA CAPTURE:	TICK COUNTING SHEET	DAY: 133

Sampling date	25-June-2018
Village name	Maserumo
GPS household	24° 54’ 50” S and 29° 45’ 32”W
Name of owner	Sekgala M.
Total number of goats	16
Number of sampled goats	5
Number of ticks counted	19
Tick tube(s) ID	5

Treated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	0	0	0	0	0	
Armpits	0	0	0	0	0	
Back legs	0	0	0	0	0	
Udder/scrotum	0	0	0	0	0	
Ears	0	0	0	0	0	
Tail	0	0	0	0	0	
Body	0	0	0	0	0	
Total ticks	**0**	**0**	**0**	**0**	**0**	**0**

Control untreated group

	Goat 1	Goat 2	Goat 3	Goat 4	Goat 5	
Front legs	1	0	0	1	0	
Armpits	0	2	2	1	0	
Back legs	0	0	0	0	1	
Udder/scrotum	0	3	0	2	0	
Ears	0	0	0	0	0	
Tail	2	0	1	1	1	
Body	0	1	0	0	0	
Total ticks	**3**	**6**	**3**	**5**	**2**	**19**

Comment(s)

The number of ticks generally reduces in the winter.

Form filled by: Mashifane M.K. **Date:** 25 June 2018

Appendix F: Product Details - Clout®

FOR EXTERNAL ANIMAL USE ONLY
CLOUT® Reg. No. G1447 Act 36/1947 N-SR0885
POUR-ON FOR CATTLE, SHEEP AND GOATS

INDICATIONS:
CATTLE
• Controls ticks
• Controls stable flies, horn flies, cattle louse flies and nuisance flies e.g. house flies
• Kills lice (biting and sucking)
• Protects against blackflies
SHEEP AND GOATS
• Controls Karoo paralysis, bont-legged and red-legged ticks
DOSE: Cattle - 1 mℓ/10 kg Sheep and goats - 1 mℓ/5 kg
STORAGE INSTRUCTIONS
Store away from food and feed.
Tightly reseal the container if all the contents have not been used.

WARNINGS

• DO NOT USE ON CALVES/LAMBS UNDER 1 MONTH.
• NO WITHDRAWAL PERIOD FOR MEAT AND MILK.
• Avoid contact with product.
• Keep out of reach of uninformed persons, children and animals.
• Although this remedy has been extensively tested under a large variety of conditions, failure thereof may ensue as a result of a wide range of reasons. If this is suspected, seek veterinary advice and notify the registration holder.
• N.B. Pyrethroid pour-on may cause irritation. Treat a small group of animals first and observe for an hour before treating the rest.

DIRECTIONS FOR USE: USE ONLY AS DIRECTED
Cattle
• **Ticks, flies and lice:** 1 mℓ/10 kg.
Apply along the backline, from the shoulder to the root of the tail using measuring dispenser or any other suitable applicator.
In case of heavy brown ear or blue tick challenge, apply Clout from the base of the skull to the root of the tail.
Treat at regular intervals according to tick challenge. The longer the product is regularly applied, the more the tick population is reduced.

It will then become possible to apply at intervals greater than 7 days.
• **For blackfly (*Simulium*)** apply also to ears and underline. Repeat as required according to challenge.
• **For population control of nuisance flies and stable flies**, use 2 - 3 treatments at weekly intervals. Thereafter use according to challenge.

Sheep and goats
Dosage for optimal residual action – 1 mℓ/5 kg.
Environmental conditions and parasite challenge can influence treatment intervals.
• **Karoo paralysis ticks:** Divide the full dose per animal into 4 equal parts. Apply one part onto each axillae and groin region, whilst the animal is turned over in a sitting position.
    • Angora goats - repeat after approximately 2 weeks.
    • Dorper sheep - repeat after approximately 4 weeks.
    • Merino sheep - repeat after approximately 6 weeks.
• **Bont-legged and red-legged ticks:**
The dosage should be divided into 3 equal parts and applied to the axillae, groin and anogenital regions.
Treat sheep at regular intervals of approximately 3 - 4 weeks or as necessary, according to tick challenge.
For crippled sheep apply 1 mℓ to the affected hoof/fetlock.

PRESENTATION: 200 mℓ, (500 mℓ, 1 ℓ, 5ℓ, 20ℓ)

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