A review of the role of pheromones in the control of *Amblyomma* ticks on livestock

By

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Declaration

I, Shaun Steven van den Hurk, 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 has not been previously submitted by me for degree purposes at another tertiary institution.

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List of Abbreviations

A. hebraeum	***Amblyomma hebraeum***
A. variegatum	***Amblyomma variegatum***
AAA	**Attraction-Aggregation-Attachment**
AAAP	**Attraction-Aggregation-Attachment Pheromone**
CO2	**Carbon dioxide**
H. rufipes	***Hyalomma rufipes***
LWG	**Live-weight gain**
R. microplus	***Rhipicephalus microplus***
DDT	**Dichlorodiphenyltrichloroethane**
PVC	**Polyvinyl chloride**
2,6-DCP	**2,6-dichlorophenol**
2-NP	**2-nitrophenol**
MS	**methyl salicylate**
R. evertsi evertsi	***Rhipicephalus evertsi evertsi***
R. zambeziensis	***Rhipicephalus zambeziensis***
R. decoloratus	***Rhipicephalus decoloratus***
R. appendiculatus	***Rhipicephalus appendiculatus***
H. irritans	***Haematobia irritans***

Summary

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Abstract:

As external parasites of livestock, ticks of the genus *Amblyomma* have a significant impact on animal health and production globally. Furthermore, *Amblyomma* ticks are vectors of diseases affecting livestock, but humans as well. *Amblyomma variegatum* and *Amblyomma hebraeum*, both found in Southern Africa, are vectors of heartwater (*Ehrlichia ruminantium*) and African tick bite fever (*Rickettsia africae*) affecting ruminants and humans, respectively. Beyond the current impact on animal and human health, there is potential for even more significant effects on animal production if the geographic range of these tick-borne pathogens will shift and expand beyond the traditional endemic areas.

A literature study was performed to evaluate the constituents of the attraction-aggregation-attachment (AAA) pheromones emitted by *Amblyomma* ticks and their effects on the behaviour of these ticks. Previous applications of these pheromones in tick control were studied and the difficulties experienced in field studies evaluated. Here, a novel method of tick control on cattle is proposed, based on the use of a legband impregnated with pheromones and acaricides, to attract and kill *Amblyomma* ticks. Specific recommendations are made to assess the efficacy and feasibility of such a control device under field conditions.

Chapter 1: Introduction

The agricultural sector is one of the most important industries for human development globally. Ticks represent one of the most important and significant parasites affecting livestock production globally. Beyond their significance for animal health and production, as vectors of diseases, ticks have a profound effect on the health and well-being of humans as well. *Amblyomma* ticks, forming one of the genera in the family Ixodidae, are numerous and a number of these ticks act as vectors of some of the diseases affecting livestock production such as heartwater [1, 2].

Amblyomma ticks are distributed across many areas of the world, and their impact on livestock health and production is pronounced [1, 3]. Chemical acaricide compounds are used in large amounts annually as there is still a dependence on them for traditional approaches of regular treatments of livestock with acaricide compounds to get these animals free of ticks. These traditional systems are becoming increasingly under-question as they are identified as labour-intensive, high-cost and resource intense, and unfavourable to the environment. These concerns are becoming more pronounced as evidence of acaricide-resistance in tick populations is expanding in recent times [2, 4, 5]. There is a great need for novel and innovative methods of tick control that address the current concerns and can be used on a large-scale basis.

It is understood that *Amblyomma* ticks have a unique set of behaviour that is underpinned by a set of pheromones emitted by fed male ticks. Through a review of literature, the aim is to explain developments that have occurred in the field of the pheromones of *Amblyomma* ticks, and then to further identify the pheromonal constituents and their concentrations in these ticks.

The aim is to further investigate the possibilities of pheromones in tick control measures, and to explore the current limitations that may exist in this area. This knowledge will be used to propose innovative, effective, and practical tick control measures based on pheromone-based techniques that can be developed and implemented for *Amblyomma* ticks on livestock.

The proposed control measures should be based on the foundation of a technique that is efficacious, simplistic in design and easy to implement. They should ideally be cost-effective and thus suited to large-scale use. It is believed that further research and development into such control measures could yield a device that would go a long way to enhance our methods of control against ticks affecting livestock. Such a device would be widely adopted and used by livestock owners as it would help to simplify their husbandry, while protecting their animals against disease and reducing the high losses incurred as a result of ticks and through the use of expensive tick control measures.

Chapter 2: Literature Review

Introduction

Ticks present a significant global health challenge for human and animal health alike; both through their direct health effects as parasites and vectors of infection, and further through their impact on livestock production and consequent food security [1]. Some of the most important tick-borne diseases affecting domestic ruminants are anaplasmosis, babesiosis, heartwater (previously cowdriosis) and theileriosis; all of which have the potential to bring about devastating health effects in livestock production units, particularly in unexposed animals [1].

With a pronounced increase in prevalence in Western Europe and the United States of America in recent times, tick-borne zoonotic diseases such as Lyme borreliosis have gained a great deal of attention and research [2]. Along with the significant effect that ticks have on global health comes the need to identify effective methods to mitigate such negative effects imposed by ticks and to enhance the production and economic viability of livestock production units. The topic of tick control has been a field of study for a long time, from the pioneer stages around 1901 when Sir Arnold Theiler and colleagues were attempting to gain control of East Coast Fever in cattle in South Africa, to more modern times where researchers are still seeking novel means of economically decreasing tick burdens or livestock with measures such as anti-tick vaccines [2].

Impact of Tick Infestation:

Ticks have been identified as being the most important pests of cattle and other domestic livestock worldwide from an economic standpoint [6]. The impact that ticks have on livestock and production is highly variable and difficult to quantify accurately. This is largely to do with the fact that such impacts observed will be determined by a number of different factors from the species and breed of livestock in question, the production system in place, the prevailing climatic and environmental conditions, tick burden experienced and the species of ticks present, to name a few. Furthermore, the effect on production and the economic impact of ticks can be identified as coming from both the direct effects of the ticks on the host, as well as from the indirect effects of the ticks (such as diseases transmitted by the ticks as vectors) [1]. The effects of ticks on humans and animals alike are largely determined by the biology and ecology of the tick species in question. Differences seen in the anatomy, life cycle, host preferences, predilection sites, geographic distribution and seasonal variations will result in unique interactions between these parasites and the humans and animals that may act as hosts for these ticks at different stages.

The ticks of primary focus in this study are ixodid ticks of the genus *Amblyomma*; more specifically the Southern African bont tick (here forth referred to as the bont tick), *Amblyomma hebraeum,* and the tropical bont tick, *Amblyomma variegatum*, being found in Southern Africa. Beyond their presence in Southern Africa, these species of tick are further implicated in the transmission of *Ehrlichia ruminantium* the causative agent of heartwater disease. Heartwater is a serious disease affecting ruminants and is of global significance both through the current impact on animal health and production.

Amblyomma ticks are three-host ticks, which means that the tick leaves the host after each of the different life stages of the tick (larva, nymph and adult) and therefore this can take place on three different hosts. It should be clear that the development of the ticks and thus their interactions with their hosts will be influenced by a multitude of factors (especially environmental factors) and that their life cycles may vary somewhat according to the prevailing conditions. The development period of the different life stages of the ticks when not attached to a host can be relatively long. However, the life cycle is usually around the period of one year for *A. hebraeum* ticks.

Certain climatic conditions may favour different life stages more than the other, and as such, it is seen that adult ticks of *A. hebraeum* are more numerous in the warm and wet summer months. In comparison, larvae are typically found in higher numbers in the colder dry months of late autumn and winter. Subsequently, the nymph stages are usually found in greatest numbers in the late winter and spring months. With this in mind however, certain areas of Southern Africa which may be considered more temperate (such as Mpumalanga, Limpopo, KwaZulu-Natal Provinces and South Zimbabwe), there is less of an impact from winter and the colder months and as such the life cycle of the *A. hebraeum* is observed as continuous with no distinct seasons. Beyond the differences in conditions that are favoured by the different life stages, *Amblyomma* also have distinct preferences for certain climatic and environmental conditions. The *Amblyomma* ticks are dependent on moisture and warmth for their survival, and as such, they require bush and scrub and do not survive the open grassland. These factors lead to relatively well-defined distribution zones for the ticks.

The different life stages of ticks often have distinct host preferences, and predilection sites on their preferred hosts and these preferences may even differ slightly between different species within a genus of ticks. Nevertheless, it is important to note that different life stages of ticks may be, and most commonly are, found on the same animal host.

It is seen that *A. hebraeum* and *A. variegatum* have rather similar host preferences and predilection sites on these hosts. The larval and nymph stages may parasitize a range of hosts including large and small mammals (including domestic livestock), birds, and tortoises; but are only seldom found on murid rodents. The predilection site of the larvae is the muzzle (face), neck, dewlap and feet on domestic ruminants. Nymphs will attach most commonly to the feet and additionally are frequently found in other predilection sites such as the legs, inguinal and perineal areas, sternum and the neck of domestic livestock.

Adults of *A. hebraeum* are typically found on larger domestic and wild ungulates, where they prefer hairless areas and are frequently found under the tail, around the perineal region, the axilla and inguinal region and more ventrally by the udder and scrotum and ventral abdomen and even the dewlap. Sheep and goats may be different where they often attach and remain attached around the feet. It is a somewhat characteristic feature of *Amblyomma* ticks to see the ticks attached in large clusters (often consisting of both adults and nymphs) around the peri-anal region.

Adults and nymphs are often seen to be "hunters" as they will actively seek a host within the vicinity and can be seen to scurry after these potential hosts in a field; this observed behaviour is further mediated by a combination of host factors and pheromones elicited by the ticks. In this active host-seeking behaviour of the nymphs and adult ticks they typically attach to the host by climbing up the hooves and attaching onto and around the feet; whereafter they may climb up the legs or migrate to other areas on the animal.

This primary site of attachment around and between the hooves is a common and significant cause of pathology, causing damage to the tissue and a tract for secondary infection. Larvae differ from nymphs and adults with their method of attaching to hosts, and they are found to ascend vegetation and thereafter will infect passing hosts.

Direct effects:

Direct effects of the tick on the animal go beyond the effect of "blood loss" and negative effects on the immune system of the animal. Direct effects include the impact that the presence of ticks and tick burden has on the growth of an animal and the stress induced upon animals with such tick worry or burden.

Moreover, direct losses may be incurred through damage to hides caused by tick bites that will reduce the market and value of such [7]. Estimated losses to hides have been quoted as between 20 and 30% as a result of tick bite damage [3]. Ticks can further directly induce negative health effects through bites to soft tissue of animals, especially where secondary bacterial infection sets in, which may lead to abscessation, mastitis and lameness; all of which may carry long-term effects or a grave prognosis for affected animals in a production system. This holds particularly true for tick species such as *Amblyomma* and *Hyalomma* with long mouth-parts and helps to illustrate the varied effects that different tick species can have on an animal and production system [2].

In a recent study conducted in South Africa, the damage caused by ticks to scrotums of bulls in communal farming areas was investigated [8]. The level of tick control on these animals was not very high, and it was noted that treatments for *Amblyomma* and *Hyalomma* tick species were probably too infrequent, thus resulting in increased tick burdens on animals. In this study, it was observed that thirty-five out of the thirty-eight bulls examined demonstrated severe scrotal and preputial lesions which were attributed to the bites of adult *A. hebraeum* and *Hyalomma rufipes* ticks.

It is very telling to note that most of the bulls tested had poor quality semen on evaluation and that this was linked with the scrotal lesions on examination. Of the bulls that were examined in this study, only three of the thirty-eight bulls demonstrated good semen on evaluation. Most remarkable is the fact that these three bulls had been recent introductions to the area and did not display the same severe scrotal lesions as the other bulls. The data from the study was suggestive of a causal relationship between the bulls with poor semen quality and scrotal lesions as observed. It was concluded that the damage done to genital areas as a result of ticks had a greater effect on the fertility of these communal bulls than contagious diseases [8].

This goes to demonstrate the effect that direct damage caused by ticks can have on animals as well as production systems. With the decreased fertility caused in these bulls, the entire production system will function sub-optimally and thus one could consider the impact of "potential losses" caused from these ticks from reduced fertility and consequent decreased calving rates.

Norval et al. [9] estimated a live-weight gain (LWG) loss of up to 10 grams per day in Africander steers in Zimbabwe as a result of infestation with *A. hebraeum* ticks. These losses include the effect of the tick feeding as well as the challenge. It has been suggested that exposure to ticks may be just as important as the number of ticks that survive and feed upon hosts. Through a proposed mechanism of hypersensitivity to ticks, it was seen that cattle that are from breeds considered as tick-resistant breeds were just as stressed from the sensitization of ticks at infestation than the cattle from less-resistant breeds were to the infestation and feeding of a greater number of these ticks [9].

Additional studies have suggested that an animal with an average tick burden of 40 ticks per day could lose up to 20kg per year in weight [3]. Thus, amounting to rather significant losses within a herd and farming operation where animals experience a high tick burden and where control measures are not carried out on a regular basis. These losses can have a real impact on a farming enterprise when one observes this from a herd and population perspective, and it is clear that they necessitate the implementation of effective tick control strategies.

Indirect effects:

Indirect health effects of ticks are largely related to their role as vectors of many important diseases, and livestock diseases in particular as previously mentioned. Diseases such as heartwater (*E. ruminantium*), and theileriosis (*Theileria spp.)* are diseases of livestock and can result in illness, debilitation and high rates of mortality.

With such a multifactorial concept, it is hard to evaluate and quantify all aspects; nonetheless, one should be cognizant of the presence of other areas that may carry an impact on production. These may include the loss and impact upon replacement stock and genetics within the herd as animals die or experience reduced growth in early development secondary to ticks or tick-borne diseases [10, 11]. Many of the studies conducted focus upon the impacts of a specific tick on livestock performance to aid clear evaluation and quantification of such impacts, although in the field animals will usually encounter a variety of tick species at the same time. Thus, the estimations provided may, in fact, under-report the true impact of ticks on livestock production. It is further important to keep in mind that the cost and impact of ticks extends beyond the effects of the ticks on livestock themselves, but also to the costs and burden incurred through control measures implemented by livestock producers and state services alike [5, 10, 12]. Tick control often relies upon the widespread use of expensive chemical acaricide compounds, and very often, these compounds are imported into countries at very high costs.

Current tick control:

Tick control is a rather dynamic field, and there has been a great deal of research done in this line to identify new and innovative tick control measures that are effective as well as economical in a practical setting. Two broad categories of tick control can be described as chemical and non-chemical control measures.

Non-chemical control:

Non-chemical control measures include a number of different strategies such as pasture control measures, grooming, ethnoveterinary therapeutic practices, anti-tick vaccination, genetic adaptation and selection, and other similar tick control measures that do not make use of chemical compounds [3, 13]. Pasture control measures include a variety of methods that often display a large degree of disparity in their efficacy which may be somewhat questionable; such methods include pasture burning, resting or removal of livestock from pastures for a period of time (rotational grazing), and removal of plant material or stripping of pastures [2, 3].

Anti-tick vaccines and vaccines developed to target tick-borne diseases are an area of increased interest and on-going research in recent times. There have been great advances in the fields of biotechnology and molecular methods to allow for easier and more efficient genetic typing, identification and processing towards the development of targeted vaccines [13, 14]. Commercial vaccines that target ticks by making use of a recombinant *Rhipicephalus microplus* Bm86 tick gut antigen have been developed, namely GAVAC and TickGARD [15, 16]. Botanical acaricides and pesticides are another field of development and increased research. Many studies have been done to test the effects of various compounds from a variety of plants of different species on ticks; and as such, many compounds illustrating positive results as an alternative to synthetic chemical acaricides have been identified [13, 17, 18].

Chemical control:

Chemical tick control includes any control method that makes use of synthetic chemical acaricides. Chemical acaricides, as has been previously mentioned, have been in use for many decades and have formed the mainstay of ectoparasite and tick control strategies since their adoption many decades ago [2, 19]. Techniques employed for chemical control include the use of plunge dips, spot-on or pour-on treatments, systemic injections, and more recently devices impregnated with acaricide (with or without mediating tick pheromones). Additional techniques involve environment-targeted acaricide use such as widespread spraying of DDT in the past or the more recent development of "TickBot" [19, 20].

Synthetic chemical compounds and classes that have been used for tick control include organophosphates (outdated and mostly out of use now), amidines (amitraz), synthetic pyrethroids, and macrocyclic lactones to name a few [5, 21, 22]. The different groups of chemical compounds act through a variety of mechanisms on ectoparasites and ticks. Many of the commercial acaricides historically have been highly effective when they are used correctly in a susceptible population. They usually result in visible effects on tick burdens of animals within a short time after use of the product. Additionally, by inducing a reduction in tick burdens on livestock, they allow the animals to better cope with inherent burdens and to achieve improved production. The major concerns related to chemical acaricides are related to their effects on environmental, animal and human health; as well as the very significant threat of increasing resistance of ticks to the compounds [3, 4].

Although the use of these compounds was adopted many years ago, they still serve as the backbone of most tick and other external parasite control programmes. Most producers make regular use of acaricides through any number of the control methods mentioned in isolation or combination with one another.

In the case of plunge dipping, this requires the use of specialised equipment that has to be built at great cost to handle large numbers of cattle that are typically brought through these setups. Thus, it is important to consider these equipment costs when evaluating a control measure and the feasibility of that system in the associated conditions.

Contrarily, acaricide impregnated slow-release devices have provided the ability to provide long-acting chemical control of ticks with the use of very little equipment and labour inputs. These devices exist in several forms but one of the most frequently observed, and that has been available for a number of years for livestock is the use of impregnated ear tags [23]. However, there is a need to reconsider the widespread use of these chemical acaricides and to work towards reducing the dependence on these traditional methods of use.

Historical overview of tick pheromones and their use in tick control:

Semiochemicals

Pheromones fit into a broader category of chemical compounds known as semiochemicals. Semiochemicals are secreted by animals and alter the behaviour of other individuals or animals, and this effect that they have on behaviour determines how the semiochemicals are classified [24]. The four main categories of semiochemicals are pheromones, kairomones, allomones, and synomones. Of these four mentioned the last kind, synomones, have not been demonstrated to carry much value to ticks and potential tick control strategies [25].

Kairomones

Kairomones are a type of semiochemical that is secreted by an individual of one species and brings about a response in other individuals of a different species; this interaction and response achieved is to the benefit of the recipient of the kairomone [26]. Certain kairomones are released by or found naturally on hosts (such as on skin) and help to excite and attract ticks. There are a number of different compounds that act in this regard, such as carbon dioxide, ammonia, lactic acid, and squalene.

However, it is important to note that not all compounds (kairomones) result in the same response from all species of ticks, and differences in compounds and concentrations present may in fact influence choice of preferred hosts for ticks [25]. It is further valuable to consider the various effects that the different compounds may have on the direct behaviour of ticks. Carbon dioxide, for example, is seen to act as a sort of generalised excitant factor for ticks and while it enhances the activity of ticks in an area, it may not provide "direction" as seen by pheromones [27]. It is thus evident that kairomones are of value when considering tick control products based on pheromones, as the presence of certain kairomones is often required to achieve the desired effect and response from ticks (the kairomones serve as additional stimulation).

Allomones

Allomones are semiochemicals that are released by an individual of one species and that have an effect on the behaviour of individuals of another species, however contrary to kairomones, this effect achieved is to the benefit of the individual that emitted it [28]. In this way, it is seen that a compound, such as squalene, can function additionally as an allomone where it is often secreted by ticks in response to and as a deterrent against attack by ants and other predators.

Pheromones

Pheromones are chemical compounds that are secreted outside of the body of an animal of a species to affect the behaviour of another animal of the same species [29]. The pheromones of ticks have been classified into four different groups; sex pheromones, primer or fecundity pheromones, arrestment pheromones, and attraction-aggregation-attachment (AAA) pheromones [30]. It is of value to understand the different behaviours that these pheromones mediate as a means to establish the benefits and results that can be achieved through their inclusion in a pheromone-based control measure. In this regard, there are a number of proposed strategies and control products that make use of these pheromones in varying manners and it is clear that there is a great opportunity for the development of innovative techniques.

Arrestment pheromones, for example, were named such as they result in akinesis and cessation of other activity in ticks when they come into contact with these pheromones [31]. These pheromones have consequently been put to use notably in products containing a sticky, oily substance and an acaricide (permethrin) used to spray vegetation to kill ticks in the environment [30]. The ticks that would ordinarily climb such vegetation would then cease activity when in contact with this arrestment pheromone and would thereby be exposed to higher levels of the acaricide and die. The results of test studies showed that the use of arrestment pheromones improved the achieved mortality rate of the product to 95% from 65% achieved with the mixture containing the acaricide without the pheromone [25, 30].

Attraction-Aggregation-Attachment pheromones

Another group of pheromones that have gained high levels of attention and research into their potential uses in tick control devices and strategies are the attraction-aggregation-attachment (AAA) pheromones (or AAAPs). Attraction-aggregation-attachment pheromones are produced by adult male ticks of some species of the genus *Amblyomma*, after feeding on a host for several days [32].

AAAPs are produced by the Southern African bont tick and the tropical bont tick, and it is these unique pheromones and the behaviour that they elicit in these key tick species of interest that engenders them of great importance to this study. The pheromones are used by unfed nymphs and adult ticks as a means to locate a viable host that would be suitable for feeding, and even more specifically to help direct these ticks to suitable sites of feeding [33]. In fact, it has been noted that ticks will rarely attach to a host that has not previously been parasitized by pheromone-producing fed male ticks. While adult females will not attach and feed on hosts without the presence of these pheromone-producing male ticks [34].

By providing direction to suitable feeding sites on a host, ticks are able to aggregate and attach to areas that are groomed less effectively and where they can stay attached and feeding for extended periods [35]. The AAAP causes the unfed ticks to aggregate in these suitable sites around already attached feeding males, and in this way form the characteristic clusters of these tick species. This induced aggregation of male and female ticks facilitates mating, and it is proposed that an additional function of the AAAPs is to promote conspecific mating of ticks [32, 36]. This is particularly pertinent as while *A. hebraeum* and *A. variegatum* have mostly distinct geographic ranges, there are a few small areas of overlap between the two species. It has been noted experimentally that even though it is not preferred, when confined together ticks of these two species will interbreed; however, the offspring of this mating are unviable [37]. With this in mind, it has been proposed that the AAA pheromones may help ticks to distinguish the alternate species through subtle differences in the pheromone compositions and thus avoid interspecific mating between the tick species [32].

The attraction-aggregation-attachment pheromones have been an area of investigation and experimentation from a number of very notable researchers in the field such as Norval, Rechav, Sonenshine and Yunker (to name a few) for many decades; with intensive research of these pheromones underway from as early as the 1970s. As such, there has been considerable growth in our knowledge and understanding of these compounds and the possible implications and uses thereof. A large proportion of these studies was aimed at identifying the composition of these pheromones and the behaviour that may be elicited by the pheromones, as well as the various individual constituents between different ticks.

It is important to recognise that researchers have identified a great deal of variation in the day to day production of different pheromone constituents and between individual ticks [38]. Further still, there has been a degree of variation in some elements between the results of different studies and researchers, with the function and even the presence of certain compounds being placed into question. This illustrates the notion that this area should be seen as a developing science and that further studies would be essential to provide a higher degree of certainty on the precise components and concentrations of these compounds at different periods and between different tick species where this is relevant. This is further demonstrated by the relative absence or deficiency of data for other *Amblyomma* tick species in Africa that have been demonstrated to make use of attraction-aggregation-attachment pheromones. Other *Amblyomma* ticks that have demonstrated these pheromones are *A. lepidum*, *A. gemma*, *A. marmoreum*, and *A. maculatum* [39, 40]. However, more recent studies and experiments that have followed the developments in this area seem aimed at identifying a response to products including components of AAAPs rather than identifying the precise composition of AAAP in these species and any interspecies variations that may be present.

Composition of AAAP

In *A. hebraeum* and *A. variegatum*, there are only a few components of the attraction-aggregation-attachment pheromones that have been repeatedly demonstrated as naturally present in ticks and responsible for inducing a behavioural response. The primary components of the AAA pheromones in this regard are 2-nitrophenol (also called o-nitrophenol), nonanoic acid, methyl salicylate, 2,6-dichlorophenol, and benzaldehyde [32]. However, of these, benzaldehyde has only demonstrated an effect on behaviour in ticks of *A. hebraeum*. Further still, 2,6-dichlorophenol is a well-documented sex pheromone of ticks, and as such, it may not be pragmatic to strictly classify this as a component of the AAA pheromones [32, 42, 43].

As mentioned, studies have demonstrated some differences in the behavioural effect induced, the different components, and the concentrations of these components in the two tick species of interest. In a series of papers, Norval and colleagues investigated a number of suspected and known constituents of the AAAPs for the resultant behaviour that was induced on ticks namely attraction, aggregation and attachment responses in these ticks [36, 43-45].

AAAP: Long-range attraction

First looking at long-range attraction factors in these ticks, the researchers investigated known or potential components of AAAP at the time. The ticks were first activated by carbon dioxide and monitored for their attraction towards filter paper impregnated with the test compounds 4 metres away [43].

Adult ticks of *A. hebraeum* were attracted towards six of the nine compounds tested, namely benzaldehyde (BZ), 2,6-dichlorophenol (2,6-DCP), heptadecane (HD), methyl salicylate (MS), nonanoic acid (NA), and 2-nitrophenol (2-NP).

Adult ticks of *A. variegatum* were attracted by four of the nine compounds tested, in 2,6-dichlorophenol, methyl salicylate, nonanoic acid, and 2-nitrophenol. From these results, it was seen that 2-nitrophenol was the most important compound for attracting adult ticks of *A. hebraeum*, and methyl salicylate and 2-nitrophenol were the most important for attracting *A. variegatum* adults.

Further still it was seen that nonanoic acid and 2-nitrophenol attracted significantly more female than male adult ticks of *A. hebraeum.* As mentioned, 2,6-dichlorophenol is well-documented compound that occurs widely as a female produced sex pheromone with a male-attractant effect.

Consequentially it is of little surprise that the compound 2,6-dichlorophenol was seen to attract more male than female adult ticks in this study [43].

AAAP: Attachment

A further study was then performed using the same known or potential components of AAAPs for their stimulation of attachment in adult ticks, and responses were noted in a number of the compounds that were tested.

Attachment was stimulated in adult male *A. hebraeum* by eight of the compounds tested; benzaldehyde, benzyl alcohol, 2,6-dichlorophenol, heptadecane, 2-methyl propanoic acid, methyl salicylate, 2-nitrophenol, and salicylaldehyde. From these compounds, the strongest attachment responses (>70%) in these ticks were observed from benzaldehyde, benzyl alcohol, 2,6-dichlorophenol, and 2-nitrophenol.

A. hebraeum adult females demonstrated attachment to six of the compounds tested in this study, namely benzaldehyde, benzyl alcohol, 2,6-dichlorophenol, 2-methyl propanoic acid, 2-nitrophenol, and salicylaldehyde. The most considerable attachment response was seen to 2,6-dichlorophenol (52%), followed by 2-nitrophenol (26%); the response to extracts from both species of pre-fed male ticks was similar at around 13-16% [44].

The results in the adult males of *A. variegatum* were slightly different where attachment was induced by seven of the tested compounds; benzaldehyde benzyl alcohol, 2,6-dichlorophenol, heptadecane, methyl salicylate, 2-nitrophenol, and salicylaldehyde. The compounds that produced the greatest response (>50%) were methyl salicylate, and 2-nitrophenol; however, the greatest attachment response was seen towards extracts of pre-fed male *A. variegatum* ticks.

Whereas adult female ticks of *A. variegatum* only demonstrated weak attachment responses (≤7%) to two of the compounds tested, namely 2-methyl salicylate and 2-nitrophenol. Interestingly it was further seen that attachment responses were induced in males of both species and female *A. hebraeum* ticks, by extracts of prefed *A. hebraeum* and *A. variegatum* adult males. However, adult female *A. variegatum* ticks were only induced to a weak attachment response (7%) by extracts from prefed male *A. variegatum* ticks [44].

These weak attachment rates that were noted in the female ticks in this experiment was attributed to a difference in behaviour between sexes of *Amblyomma* ticks, where females seem more dependent on physical stimulation prior to attachment than male ticks. This derives from noted behaviour of female *A. hebraeum* ticks usually being clasped by attached pheromone releasing males before attachment; while males attach in the absence of such stimulation.

AAAP: Aggregation

The third study in this series was aimed at evaluating known and potential components of the AAA pheromone for their ability to elicit an aggregation response in *A. variegatum* and *A. hebraeum* ticks. In this study, a further compound, phenylacetaldehyde, was tested after it had recently been tentatively identified in the extracts of fed *A. hebraeum* male ticks [36].

As has been previously mentioned, this aggregation behaviour and response is an important aspect of the ecology of these ticks and is thought to play an important role in the selection of suitable feeding sites and encouraging successful and conspecific mating where these tick species may overlap. As such, the components responsible for eliciting this behaviour, together with any inter-species differences that may exist is of experimental interest.

In this study, it was noted that aggregation was elicited in adults of *A. variegatum* by four of the compounds tested, namely 2,6-dichlorophenol, methyl salicylate, 2-nitrophenol, and phenylacetaldehyde. Of these compounds the first three listed function as long-range attractants and attachment stimulants, while phenylacetaldehyde served only to induce aggregation in these ticks.

An interesting observation in these *A. variegatum* ticks was that while an aggregation response was noted when testing these identified compounds individually or as mixtures; when these mixtures contained compounds that have not been identified in fed male *A. variegatum* no aggregation occurred. It was thus hypothesised that one or more of these compounds might be an inhibitor or deterrent of aggregation for *A. variegatum* ticks [36].

This was further defended by the fact that while there was a strong aggregation response to the extracts of five prefed *A. variegatum* males, there was only a weak response elicited by the extracts of fifty prefed *A. hebraeum* male ticks. This could be attributed to the fact that the extracts of these fed *A. hebraeum* ticks may contain increased concentrations of compounds such as benzaldehyde, heptanoic acid and 2-methyl propanoic acid, which could act as inhibitors of aggregation for *A. variegatum* ticks. On the other hand, in this study, the only individual compound tested that caused unquestionable aggregation of *A. hebraeum* adult ticks was phenylacetaldehyde.

It was observed that while other compounds in benzaldehyde, 2,6-dichlorophenol, methyl salicylate, and 2-nitrophenol caused attraction of the ticks, they did not stay still and form characteristic aggregations at the source.

For *A. hebraeum* ticks it was noted that the greatest aggregation response was elicited towards fifty live recently-prefed (removed from cattle) conspecific males. While a considerable aggregation response was brought about by the extracts of fifty prefed male *A. hebraeum* ticks and no aggregation or a slight response was elicited by the extracts of five prefed conspecific male ticks [36].

It is interesting to note that in this paper, phenylacetaldehyde was identified as an important component of the AAAP and was identified as serving an important function for the aggregation response of these species of ticks. However, in a further study aimed at identifying the pheromonal composition of these two tick species, Price et al. [32] noted that the presence of phenylacetaldehyde could not be confirmed in *A. hebraeum* and *A. variegatum*.

With this doubt as to whether phenylacetaldehyde is naturally present in these tick species, it can be questioned whether the aggregation behaviour that was induced was another non-specific response to a phenolic compound (as seen with the attractants) [33, 46]. Following this one could perhaps further question whether the patterns observed in the aggregation behaviour of *A. hebraeum* ticks in response to the compounds tested was as a result of relative concentrations that were present, as opposed to that naturally found in prefed ticks, and particularly in prefed live ticks.

AAAP constituents of *A. hebraeum* and *A. variegatum*

In the study performed by Price et al. [32], the compounds 2-nitrophenol, 2,6-dichlorophenol, nonanoic acid, methyl salicylate, and benzaldehyde were demonstrated in both *A. hebraeum* and *A. variegatum* ticks. However, from these compounds, methyl salicylate and benzaldehyde were only found in one of the two test sets from *A. hebraeum* and *A. variegatum,* respectively.

Methyl salicylate which been shown in previous studies to induce strong attraction and attachment responses in both species of ticks, was seen to be in significantly lower quantities in *A. hebraeum* (only traces were detected and sometimes not detected at all) compared to that of the tropical bont tick [32].

This contrasts to benzaldehyde, where the relative abundance is found to be much lower in *A. variegatum* ticks (it was not found in female ticks at all) than that found in *A. hebraeum*. This coupled with the fact that benzaldehyde was seen to inhibit aggregation in *A. variegatum* ticks helps to support the theory that small differences in the relative abundance of components of the AAAP between species exist and that these play a crucial role in the resultant behaviour observed in these ticks [32, 36].

In this study, 2-nitrophenol was observed as the compound with the greatest abundance in both species of ticks; however, the relative abundance of this was significantly lower in *A. hebraeum* ticks than in the interspecific ticks. These observations correspond with those of Diehl et al. [42] who reported emission rates of 2-nitrophenol between 0.4–1.8µg/hour/tick (males and females) in *A. variegatum*, as opposed to the lower emission rates found in *A. hebraeum* ticks of 0.23-0.288µg/hour/tick. The high concentrations of 2-nitrophenol in these ticks is apt considering that it constitutes a very important component of the AAA pheromones. In both species 2-nitrophenol functions as a primary long-range attractant and stimulates attachment, and in the tropical bont tick it further stimulates an aggregation response [32, 36, 43].

Price at al. [32] further identified heptanoic and octanoic acid, present only in *A. hebraeum* ticks; however, the functions of these compounds and their potential role as pheromone constituents is unknown.

Evaluating concentrations of AAAP components

While the precise constituents and associated concentrations of the attraction-aggregation-attachment pheromones, and the relevant interspecies differences, are of great value in understanding the behaviour and ecology of these ticks. The primary value and research interests exist in understanding these factors so that we may put them to use in strategic control measures against ticks.

Therefore, a practical knowledge base underpinning the main components of the AAAPs is of great benefit. It is in this light that the first guidelines relating the main chemical components of the AAAP in *A. variegatum* and the prevailing relative concentrations from Schoni et al. [47] was of particular value. The researchers identified the main components of the AAA pheromone in these ticks as 2-nitrophenol, methyl salicylate, and nonanoic acid; and they went on to report that these existed in a ratio of 2:1:8µg/tick in the extracts of fed male ticks [47].

Further research from Diehl et al. [42] in identifying these compounds and their emission rates supported these constituents; however, the values of the amounts of emitted 2-nitrophenol and methyl salicylate in the tropical bont tick was seen to vary according to the stage of feeding and consequently the ratio also changed. Further still, it has been noted that variations occur in the amount of pheromones that are emitted between individual ticks. This ratio of emitted 2-nitrophenol to methyl salicylate ranged between 2:1 and 7:1 after 12 days of feeding, with an observed mean of 4:1. In their study, they were not able to accurately quantify and get comparable values for the amount of nonanoic acid present to compare the full ratio as previously published [42]. Following on from these observations, a number of experiments and several approaches have been made at tick control measures harnessing these pheromones.

The use of AAAP in devices and field tests

As a means of identifying the potential use of these AAA pheromones in attracting ticks in field conditions, Norval and colleagues [27] tested the attraction of adult and nymphs of *A. hebraeum* and *A. variegatum* to sources of AAAP and CO2. The results obtained were very positive as it was seen that ticks were attracted to the combination of these components from as far as 25 metres away (mainly nymphs which were significantly more attracted at these greater distances). While adult ticks were strongly attracted to sources of AAAP and CO2 from distances of 10 metres away, and marked responses were still seen from up to 15 metres away in this study [27].

The promising results demonstrated by such tests of AAAPs has led to several devices centred on the concept of "attract-and-kill"; with numerous studies looking into traps that incorporate this concept, to devices that can be attached to livestock. Traps that can be placed in an area and will thereafter attract ticks to the device, where they may be exposed to an acaricide or other lethal compound to "remotely" control a tick population have been investigated.

Bryson et al. [48] demonstrated good results in attracting and trapping *A. hebraeum* ticks (adults and nymphs) over a sustained period with trap devices making use of CO2 and extracts of AAAPs. This study is particularly encouraging as a proof of concept as it was carried out over a wide range of ecological sites (where *Amblyomma* ticks are found) in South Africa, and in farming systems with a variety of management systems and differing tick burdens. This study helped to illustrate that if such a device is to be used strategically and placed in selective areas, it is possible to "target" micro-populations of ticks that may be present in an area [48].

In furthering the development of this concept, Maranga and colleagues conducted a series of experiments to evaluate the attraction of *A. variegatum* ticks to synthetic AAAP and CO2, and thereafter to these baited trap devices with an entomopathogenic fungi to establish a new attract-and-kill device without acaricides [49, 50]. The finding supported previous studies that the combination of AAAP with CO2 yielded the best attraction response (with CO2 alone being found as unattractive, and AAAP alone only mildly attractive to ticks), and it was found that specific concentrations of AAAP yielded optimal attraction.

The optimal concentrations of the synthetic AAAP components for attraction as repeated in the trap devices resulted in amounts of 1.2mg 2-nitrophenol, 0.6mg methyl salicylate, and 4.8mg nonanoic acid; and it was further questioned whether higher doses had a repellent effect on ticks (as has been previously proposed) [49, 50].

This concept was further investigated by Nchu et al. [51], who further investigated the use of semiochemicals as bait for *A. variegatum* ticks to a trap with an entomopathogenic fungi. In this study, additional compounds in butyric acid and 1-octen-3-ol were added to the AAA pheromone constituents as tested in previous studies. The study tested various concentrations of AAA pheromone, with and without these new proposed compounds and evaluated the attraction of ticks from various distances in olfactometer and semi-field studies.

The AAA pheromone consisted of a ratio of 2:1:8 of 2-nitrophenol, methyl salicylate, and nonanoic acid, respectively. It was noted that the unfed *A. variegatum* ticks were repelled by butyric acid, and as such, it was not included in the semi-field tests. Interestingly, it was once again noted that less attraction was observed at high concentrations of AAAP; and in fact, the ticks significantly avoid the source of the AAAP in the olfactometer tests when at a concentration of 1.1mg in this study.

In the semi-field test, it was noted that adult tropical bont ticks were attracted from up to 8 metres away, and that there was no significant difference in the level of attraction from 1 metre to 6 metres away from the baited device. This level of attraction was achieved from a combination of 0.088mg AAAP and 64ng 1-octen-3-ol and CO2 in the baited trap. This is in an improvement on the level of attraction that was achieved by Maranga et al. [49] where the maximum distance from which attraction was achieved was between 5 metres and 6 metres; and one could question whether this is linked to the addition of this new compound, enhanced (more accurate) concentrations of the AAAP or perhaps even prevailing climatic conditions [51].

AAAP impregnated devices

Additional studies that have been done to investigate the plausibility of "attract-and-kill" systems based on AAAPs have involved making use of slow-release impregnated devices that can be attached to animals. These devices are based on the idea of a polymer (such as polyvinyl chloride tags) being impregnated with the constituents of the AAA pheromones and an effective acaricide in a sustained release formulation. These devices can then be attached to an animal, where ticks will be attracted to the device and be exposed to lethal (effective) concentrations of the acaricide.

It is this concept of making use of such a device on an animal host for strategic tick control that is of primary interest for the researchers. There are a few obvious advantages to making use of this control measure on an animal host, including but not limited to the fact that: this is direct and targeted to control (reduce) tick burdens on animals, the extended period of tick control with sustained-release devices, and making use of animal hosts obviates the need for additional compounds (such as CO2 that may be needed in standalone traps).

Zimbabwe trial

One such study was performed with a trial that was conducted in Zimbabwe following the principle described above to test the efficacy of control of *A. hebraeum* ticks. In this large-scale field test, consisting of two three-month-long trials, polyvinyl chloride (PVC) tags that had been impregnated with AAA pheromone components and slow-release acaricide were manufactured [52].

The AAAP complex that was used was made up of 1% 2-nitrophenol, 1% methyl salicylate, 0.2% 2,6-dichlorophenol, and 0.1% phenylacetaldehyde (this is not a natural compound) per 100g of PVC (each tag weighed approximately 10g). The acaricides were impregnated into these tags in slow-release formulas consisting of 7.5% per 100g of PVC of cyfluthrin, flumethrin or alphacypermethrin.

The trials were conducted on 300 mixed-breed cattle from a herd which were separated into different paddocks for the respective test groups. The tested animals were then fitted with an impregnated tail tag based on the respective group (except for animals that were left untreated). For the first trial the test groups were flumethrin and pheromone tags (100 animals), cyfluthrin and pheromone tags (100 animals), pheromone only tags (25 animals), and untreated animals (75 animals).

In the second trial, the additional acaricide in alphacypermethrin was tested and as such the groups were: flumethrin and pheromone tags (100 animals), cyfluthrin and pheromone tags (100 animals), alphacypermethrin and pheromone (75 animals), pheromone only tags (15 animals), and untreated animals (10 animals).

Over the duration of the two trials, significant results were demonstrated in the degree of tick control achieved. The overall control rate for adult *A. hebraeum* ticks was 94.9% and 99.3% from the first and second trials, respectively. Differences were noted in the levels of control achieved by the different acaricides, with cyfluthrin achieving the best overall control rate of 98.7%, the flumethrin combination tags yielded an overall control of 94.8%, and the level of control by the alphacypermethrin combinations was significantly lower with an overall control of 55.9% achieved.

Moreover, it is interesting to note that the cyfluthrin groups demonstrated significantly fewer male ticks than the other two acaricide-treated groups in this study. It was further observed that cattle with the cyfluthrin- and flumethrin-impregnated pheromone tags demonstrated moderate control of other tick species present, with decreased tick burdens for *R. evertsi evertsi*, *R. zambeziensis*, and *Hyalomma spp*. Tick control for these other species of ticks between the two trials in this study ranged from 59.2% to 82.2% for the cyfluthrin-impregnated tags; and 46.0% to 74.5% for the flumethrin-impregnated tags. The researchers noted that unfortunately, there were insufficient numbers of blue ticks (*R. microplus* or *R. decoloratus*) present on the farm to evaluate the level of control that could be demonstrated against these species.

Investigation into the loss of chemicals from the tags indicated that there would be a 100% loss of the compounds in approximately 12 weeks and 14 weeks for 2-nitrophenol and methyl salicylate, respectively. Thus, the tags would have to be replaced on animals every three months to maintain effective control. In this study, it was reported that the retention of the tail tags was excellent for most of the study period. However, there were issues observed with the tape that was used to fix the tags to the tails as a result of different weather conditions [52-54].

Guadeloupe trial

In a follow-up study performed by Allan and colleagues, similar impregnated tags were tested for their efficacy in controlling *A. variegatum* ticks in Guadeloupe in the Caribbean [53]. This study involved a thirteen-week trial with six different treatment groups that were made up of different herds of cattle (between 12 and 20 animals each) that were kept on different pastures. Some of these pastures were close to each other and experienced similar levels of tick burdens; however, this was not the case for all the groups/pastures.

In this study each animal was fitted with two impregnated tags, one was fitted to a collar on the neck and the second was attached to the tail. The AAAP component was made of the same components in the same concentrations as the previous study; 1% 2-nitrophenol, 1% methyl salicylate, 0.2% 2,6-dichlorophenol, 0.1% phenylacetaldehyde per 100g PVC; and once again the tags weighed 10g. The acaricides that were tested in this trial were deltamethrin and cyfluthrin, included at 7% and 7.5% per 100g PVC, respectively.

The six groups of cattle were classified according to the treatment received: untreated animals (U), pheromone only tag (P), cyfluthrin only tag (C), pheromone and cyfluthrin tag (PC), deltamethrin tag (D), and lastly the pheromone and deltamethrin tag (PD). It was noted that the first four groups mentioned had pastures with a much lower tick burden than that experienced by the last two groups. As such it was decided that it would not be feasible to compare the level of control by comparing the groups, and so it was decided to base the level of control achieved on the change in tick burden of animals from prior to treatment.

In this regard it was noted that the level of control achieved in both groups with deltamethrin-impregnated tags was excellent; with a significant decrease in tick numbers from a week after placement of the tags. The tick infestations of the animals in these groups at the end of the trial were decreased by 87.6% and 68.7% by the deltamethrin-impregnated, and the pheromone and deltamethrin-impregnated tags, respectively.

In this study it was seen that the cyfluthrin-impregnated tags yielded moderate tick control; with decreases in tick density at the end of the trial amounting 42.8% for the cyfluthrin-impregnated tags, and 45% decrease achieved from the pheromone and cyfluthrin tags. This is a notable difference to the pattern and results achieved in tick control achieved by cyfluthrin in this trial compared to that seen in the previous similar trial conducted in Zimbabwe [52, 53].

Several possible reasons have been suggested for these observed differences including the fact that the underlying low level of tick burdens present on these animals/pastures before treatment may have made it more difficult to accurately assess and quantify (compare) the level of control achieved. Differences in the prevailing climatic conditions, particularly ambient temperatures and humidity, between Zimbabwe and Guadeloupe have further been suggested as possible factors to account for the variance in the levels of control achieved from cyfluthrin between these two studies. It has been noted that differences in humidity can have an effect on the relative vapour pressure gradients and consequent loss or diffusion of insecticides. Though it should be noted that the results seen by these treatments are considerable when compared with the fact that the untreated group displayed an increase in tick burden of 311.7% from the beginning of the study [53].

Another aspect of this Guadeloupe trial was to test hair samples at different areas of the animals' bodies to determine the distribution of acaricide compounds and the residual levels of these compounds across the period of the study [53]. It was seen that there was a good distribution of the acaricides across the body within two days of application of tags. There was a steady decrease of these residual levels across the body over the study period; still, it was observed that the levels of deltamethrin present were still above the effective toxic dose at the end of the study.

The concentrations of the AAA pheromone components in the PVC tags were monitored over the duration of the trial to similarly evaluate the degree of loss/retention of these compounds throughout the experiment. As expected, there were decreased concentrations of these compounds found at the end of the thirteen-week trial. Nevertheless, these were identified as being above the threshold level for behavioural stimulation. At the end of the trial, the levels of the pheromone components that were found to still be present in the tags were; 2,6-DCP 2.08mg/10g, 2-NP 1.39mg/10g, MS 2.64mg/10g, phenylacetaldehyde 0.82mg/10g.

In this trial, the retention of the tags was good, although it was noted that retention of original tail tags for the full period was decreased. The tags were attached with a different technique in this study as the researchers made use of a cyanoacrylate adhesive and metal staples to attach the tags to a neck collar and to a clump of hairs on the switch of the tail. This prevented the problems that had been experienced with failure of adhesives, albeit it was seen that tail-tags were still lost due to the hairs being pulled out of the tail.

The retention of the tags on collars was 98%; however, it was seen that the tail tags were lost at a rate of approximately 3% per week for the first seven weeks, and then 9% per week thereafter. This brings the longevity of this method of attaching the tail-tags into question as the retention rate of original tags started at 90% for the first five weeks and then decreased to approximately 55% by the end of the trial.

St. Kitts trial

More recently, in a study to evaluate the efficacy of the slow-release pheromone/acaricide tags for the control of *A. variegatum*, a twenty-one-month trial was conducted on St. Kitts in the West Indies [55]. The trial design was similar to that of Allan et al. [53] in the Guadeloupe trial; however, it was conducted on two farms making use of mixed breed cattle that were allowed to free-roam in the area for grazing.

Cattle were once again fitted with impregnated tags on neck collars and the tail switch, and these tags were replaced every three months (or when identified as lost at monthly tick counts). Within these herds two study groups were evaluated; namely “treated animals” that were fitted with tags impregnated with deltamethrin and AAAP compounds, and “sentinel animals” that were fitted with tags impregnated with AAA pheromone components alone.

The numbers of animals that were used in the respective treatment groups were changed from the proposed numbers at the start of the study. Initially, two animals from each farm were used as sentinel animals and fitted with respective tags; however, this was increased to four animals on each farm. Initially Farm A was supposed to have 11% of the animals in the “treated” group, but this became 44%

(7/16 animals) by the end of the study. Farm B had the number of animals in this "treated" group increase from the proposed 12% of the herd to 29% (5/17 animals). These changes were made approximately halfway through the study, at month ten of the twenty-one-month trial.

The AAAP compounds used in the tags in this study were the same as that used by Allan et al. [53] in their trial; specifically 1% 2-nitrophenol, 1% methyl salicylate, 0.2% 2,6-dichlorophenol, 0.1% phenylacetaldehyde per 100g PVC. Unlike previous studies, there were no comparative evaluations of different acaricidal compounds, and the respective treatment tags were impregnated with 7% deltamethrin per 100g PVC.

The level of control of tick burdens experienced in this trial was good with an overall average of 92%; with the level of control on farm B (97%) being higher than that seen on farm A (86%). This is demonstrated in an infestation rate of an average of 3.5 ticks/animal at any examination (usually monthly) on animals treated with deltamethrin-acaricide tags; compared to the average of 23.1 ticks/animal at any examination for the sentinel animals.

The areas in which the tests were carried out were noted to have high tick densities, and this was further exemplified by farm A (31.4 ticks/sentinel) which had an elevated tick pressure than farm B (15.9 ticks/sentinel). It was noted by Kelly et al. that the tick burdens seemed to increase within the second year of the study, and that the level of "environmental control" of tick populations was poor [55].

It is believed that this reduced level of control of tick densities in the environmental population is largely related to the fact that the cattle used in the trial were free-roaming, and thus they covered very large areas and were exposed to many different areas and "sub-populations" of *Amblyomma* ticks. This is further exacerbated by the fact that the areas in question were largely unkept and thick bush terrain (with many other wild hosts possible) that would be ideal for the proliferation of the tropical bont tick. Thence, it stands to reason that one could not expect the same level of control in such a setting as one may expect when dealing with a closed system of a herd kept on pastures.

Barre and Garris [56] have proposed that in the absence of reinfestation, regular acaricide application to a herd of cattle with a stocking density of two to four animals per hectare would remove all tropical bont tick in three to five weeks [55]. This goes to support the idea that the level of environmental control may have been improved by reducing the environmental range, or through increasing the number of animals within the herd that were treated (higher treated percentage) within the herd.

In this study the tags were attached in a very similar manner as was seen in the Guadeloupe trial as cyanoacrylate and metal staples were used for attachment of tags; and similarly, it was seen that most tail tags were lost through hairs being pulled out of the tail [53, 55]. The researchers described a significant loss of tail tags (neck tags that were applied were not a concern), which was made worse by cattle moving through thick bush and areas where the tags could get caught and pulled from the tail. It was noted that 38% of tail tags were lost before the full three-month period; where farm A, with thicker bush and scrub present, had 48% of the tail tags lost and farm B had 28% of the tail tags from animals lost.

The loss of deltamethrin from the tags did not appear to be very severe within this trial as the remaining levels were between 4.5% - 5.9% after the three-month period on animals [55]. This should further highlight that infecting ticks would likely have been exposed to adequate concentrations of deltamethrin to achieve effective toxic dose. Therefore, the justifications mentioned above or the possibility of acaricide resistance amongst the tick population should be brought into question if the level of environmental control is to be scrutinised.

Conclusion:

Tick pheromones have been studied for many years, and our knowledge and understanding of their constituents and uses have developed remarkably. The attraction-aggregation-attachment pheromones of *Amblyomma* ticks have been at the centre of numerous field experiments undertaken by researchers; and have demonstrated very significant results.

It is believed that the field of pheromone-based tick control measures has the potential to provide highly effective and strategic solutions in a practical setting while maintaining a cost-centred approach. Pheromone-based measures have been shown to be applicable to a number of different settings and applications. Furthermore, they have proven to be easy to initiate (user-friendly), which greatly enhances their potential uptake into control measures practised at the “ground-level”. With further development and refinement of the techniques and products that have been put forward thus far, this control strategy has the potential to become an established and widespread solution for tick-control.

Chapter 3: Methodology

A literature review study was performed with the aim of evaluating the attraction-aggregation-attachment (AAA) pheromones of *Amblyomma* ticks and their potential uses in tick control measures. The primary databases that were used for all of the literature searches were "Web of Science" and "Science Direct".

The initial search terms used were topic searches: (Amblyomma AND pheromone*), ((Amblyomma OR tick*) AND pheromone*), and (tick* AND pheromone* AND (livestock OR cattle OR sheep OR goat OR small stock)). All of these searches yielded a practical number of results to allow for the search results to be evaluated on an individual basis to determine the relevance to the scope and aims of the project. The exact numbers of results obtained from these searches was not recorded, and as such are not included. On this basis, articles were either included or excluded from these initial results.

As the importance of previous research done by Norval and Sonenshine was noted, further literature searches were performed using the following search terms: (Author: "Norval, R" AND Topic: "tick*"), and (Author: "Sonenshine" AND Topic: "tick*"). Once again, these searches yielded a practical number of results to allow for individual evaluation, as mentioned above.

Thereafter, a number of additional resources and references were identified by evaluating the references in articles read, and if deemed to be pertinent and missing from the articles present, they were added. Other smaller searches were conducted as the study continued if it was felt that more information was needed on a particular point; for example, on the economic impact of ticks.

Chapter 4: Discussion

Ticks of the genus *Amblyomma* are implicated in carrying many significant diseases of humans and animals, and their effects on production are well documented. However, the unique behaviour of some of these ticks to respond to attraction, aggregation, and attachment stimuli through the influence of pheromones emitted by fed males allows an inroad to targeted control measures of these ticks.

The behaviour of Amblyomma ticks and the function of AAAP

A. hebraeum and *A. variegatum* are both three-host ticks, and the adult ticks are found predominantly on large ungulates; and are rather adept at parasitizing domestic ruminants. The main predilection sites of these ticks are often the hairless regions of the perineal area, under the tail, around the udder/prepuce and scrotum, and the axilla. It is in these regions where they will often accumulate and form aggregations in the absence of control measures.

As previously mentioned, this behaviour in these ticks is brought about through the action of attraction-aggregation-attachment pheromone produced by these ticks. These ticks live in areas with bush and scrubs where there is abundant vegetation and organic material for them to hide underneath. The unfed adults will usually remain inactive under this vegetation or under the soil where they are protected until they are exposed to and become activated by host stimuli.

The excitation effect of carbon dioxide on ticks has been mentioned; and when these ticks are exposed to large volumes of carbon dioxide, as expelled from the breath of large ruminants, they are aroused to leave their shelter. This induces the non-specific excitation in these ticks that causes them to search for a host; the so-called "random questing" behaviour [32-34, 44]. If the animal from which the CO2 emanated is infested with fed males of the species, then these fed males should be emitting the AAA pheromone as explained.

The primary function of the AAAP is to induce long-range attraction of these unfed ticks; and thereby provide them with direction towards a suitable host. Hence, ticks can become activated, attracted, and directed towards hosts from distances as far away as fifteen metres (or perhaps further). Once upon the host, the ticks then move towards the ticks that are the source of this male-produced AAA pheromone.

Regulated by this pheromone, the movement of the ticks ceases when near the source (fed males); hereafter, they are drawn to form aggregations of feedings ticks. Aggregations are usually formed on body areas that are poorly groomed by the host, as these are most conducive to undisturbed feeding and propagation. This cessation of activity is closely followed by an attachment response which is further induced by the presence of AAA pheromones on the host's skin from these fed males. As such, the three functions of the attraction-aggregation-attachment pheromones help unfed ticks to locate and parasitize suitable hosts; and bring adults together to facilitate conspecific mating [32, 34, 36, 57].

Parasitization process of Amblyomma ticks

It has been stated that to get onto hosts and to move to the sites of attachment (predilection sites), the adults and nymphs of these *Amblyomma* ticks climb up from the feet of potential hosts. The dynamics and underlying processes relating to infestation and attachment processes of *A. variegatum* adults on cattle was investigated in a study in West Africa [58]. A number of very interesting observations were made on the underlying behaviour and processes and further still the implications that these could have for tick control strategies.

Tick numbers on cattle, the distribution of males and females, as well as the locations of attachment were observed. An essential factor in this research is that experiments were carried out with cattle that were farmed under a more traditional system of sending animals out to the paddock or field to graze during the day and then bringing them into another paddock at night. Observations were carried out at different times of the day, with fundamental comparisons being made between the distribution during the day and night, and along with different behaviour carried out by cattle. These behavioural differences were primarily aimed at comparisons in time spent lying down versus time spent standing and relating this to the distribution of ticks on the animals. From noting the tick numbers and distribution of tick infestation on cattle under several different circumstances and at different times of the day, analyses on the infestation processes could be formed.

Stachurski [58] determined that a two-stage infestation process exists in these ticks. It was noted that the vast majority of ticks found on the cattle were picked up while grazing on the pasture during the day, and that these ticks could be found temporarily (weakly) attached to the limb ends near the hooves. This distribution then changed, usually at night, when cattle were able to lie down; and then the proportions of ticks were found to be higher on the predilection sites where the ticks attached decisively (strong attachment).

Observations made indicate that when the cattle lie down, the ticks tend to detach from their temporary sites on the lower limbs and will then move towards and attach to the preferred sites of feeding in predilections sites on cattle. This movement may sometimes occur on the same animal or ticks may migrate to another animal nearby, their studies indicated that up to one-third of ticks changed hosts during this move [58]. It is noted that if animals do lie down in a field that is infested by ticks, attachment directly to predilection sites of the ticks on cattle may occur without following this two-phase model, although this is not identified as the norm.

Thus, when the animals were not allowed to lie down during the day and spent the entire day grazing in the field, most of the attached ticks (94%) were found on the feet. While if these animals spent increased time lying down or allowed to rest and lie down throughout the day, then the proportions of ticks that were found on the predilection sites increased dramatically to 37% and 54% in two of the experiments carried out.

A further thought-provoking observation was made in that there were no differences between the numbers of male and female ticks that were found attached to the distal limbs. A proposal was made as to whether host-factors from the animal alone, without the presence of AAA pheromones from fed males, was enough to induce this initial attachment of both sexes of ticks. This could be fitting when considering the responses yielded in attraction studies of ticks.

While CO2 seems to act as a generalised excitation factor, in close ranges it may provide enough stimulation for this host infestation, but the value of the host factor 1-octen-3-ol in attraction studies is telling. As previously discussed, in their studies, Nchu et al. [51] demonstrated that the addition of the compound 1-octen-3-ol to AAA pheromone components yielded increased attraction to baited traps.

However, the role AAAP was noted when analysing the movements and attachment of ticks the predilection sites. The patterns demonstrated indicate that female ticks were largely unable to attach to predilections sites in the absence of pioneer males that had first attached for a number of days (emitting AAA pheromones). While the presence of other feeding males did seem to facilitate the attachment of additional males, and thereby increase the overall proportions of these ticks on predilection sites, the presence of pioneer males did not appear a prerequisite for this attachment.

It should be said that while there is this pronounced migration of ticks from their temporary sites of attachment, the researchers did witness that a large number of ticks (40%- 50%) were lost during the night from that observed on the feet during the day. It stands to question whether these are lost as a result of host grooming behaviour, ticks truly getting “lost” as they lose direction, or as Stachurski suggested, numbers of ticks may die after expending too much energy seeking a host and this initial infestation [58].

The use of AAAP for tick control

The investigations into the attraction-aggregation-attachment pheromone of some species of *Amblyomma* ticks has led to a much greater understanding of the behaviour of these ticks as well as the pheromonal composition itself. Through many studies and experiments over the years, the components of this pheromone and the reciprocal behavioural responses from ticks can be manipulated to good effect. Devices and studies making use of components of the attraction-aggregation-attachment pheromone and host factors and other semiochemicals have demonstrated significant results of tick control.

These devices and techniques making use of AAAP are beneficial as they provide a more targeted and strategic means of tick control; especially when compared to more traditional methods. The use of factors that are known to mediate the behaviour of ticks is of benefit as it facilitates the use of the "baited-trap" or "attract-and-kill" principles. With the use of AAAP *Amblyomma* ticks can be enticed to move towards, come into contact with, or aggregate around acaricidal compounds such as traditional chemical compounds or even lethal entomopathogenic fungi [50, 55].

Benefits of AAAP-impregnated devices

Beyond the significant results in terms of the level of response and tick control achieved with the use of these devices and techniques, there are a number of other benefits to their use as well. These impregnated tags, as used in the trials in the Caribbean, are relatively inexpensive and are easy to make [53, 55]. Kelly et al. [55] reported that the cost of the tags amounted to 0.2% of the cost of the equivalent six treatments of 1% flumethrin (at the government subsidised rate) that would have been used in the absence of the tag. The respective costs of production of the tags at the time were US$0.22 for the pheromone-only tag, and US$0.46 for the deltamethrin-pheromone tag. Thus the use of these tags could help reduce the need for expensive chemical acaricides and reduce the impact of these costs on the industry.

The pheromone-acaricide impregnated tags, as used in the aforementioned studies, demonstrated high levels of tick control for the three-month periods tested [53-55]. The use of a strategy that provides good control for an extended period with a single application is highly advantageous as it is resource and user-friendly. Reducing the number of applications or treatments of animals for ticks would greatly reduce labour costs, as well as reduce the use of equipment (such as dip tanks and spray races), as well as reduce the amount of chemical acaricides used.

This carries a significant environmental benefit as there would be less environmental pollution from chemical acaricides and no need for precious water resources to dip and treat cattle as is currently the case. This is of tremendous value in many African countries where water resources can be scarce as many areas are drought-stricken. In some communal regions such as those in Bushbuckridge surrounding the Kruger National Park in South Africa, the dipping of communal livestock is carried out by the state veterinary services and there have been times when this dipping has been unable to continue due to shortage of water reserves resulting from poor rains and drought experienced (personal communication) [59].

As mentioned, these pheromone-acaricide impregnated tags are user-friendly as they have a very simple mechanism that does not require any expertise or knowledge to determine dosages or different application methods. A tag can be designed for a particular method of application and distributed accordingly; as has been demonstrated in other commercially available products for pets (Seresto® collar, Bayer Animal Health) and cattle (CARAVANAS®, Bupo Animal Health). It is in this regard of application; however, where a number of the products and trials evaluated thus far have experienced the greatest challenges.

Difficulties experienced with AAAP-impregnated devices

In the studies that have been presented from Zimbabwe and the Caribbean, PVC tags impregnated with AAAP and acaricides (individually or in combination) were attached to neck collars and the tails of cattle [53-55]. While the level of tick control achieved, as mentioned before, was good and significant results were noted; one of the preeminent challenges that was shared in the different studies was that of application and retention of the tags.

Failure of retention of tail tags, in particular, was a challenge and a possible limitation that was observed with this method of tick control. Some of the biggest issues faced with the tail tags were: failure of the adhesive tapes under different climatic conditions, constriction of the tail causing lack of blood flow to the distal tail, and the loss of hairs to which the tags were attached. The loss of these hairs was noted to largely occur from the tag getting caught in a fence or thicket and then the hairs of the tail being wrenched out. This is unsurprising when one considers that the general behaviour of cattle involves a lot of movement and flicking of the tail throughout the day.

This is combined with the fact that the cattle in the Caribbean studies were allowed to free-graze in the surrounding areas; and that these areas typically contained increased densities of thick bush and scrub (the preferred habitat of the *Amblyomma* ticks). Thus, there was a high likelihood of these tags undergoing high levels of stress and forces induced upon their point of attachment; as well as getting caught as cattle walked and grazed.

The PVC tags that had been attached to the neck collars did not seem to present as great a challenge with retention; presumably as a result of fewer challenges and stresses exerted upon them. Additionally, as a point of attachment, the neck allows a greater surface area and will be able to carry larger and heavier devices than the tail. However, when making use of an impregnated device to control ectoparasites the choice of a location of attachment should be based on the organisms that are targeted.

Different sites for attachment of slow-release devices

In a set of rudimentary tests, Beadles et al. [60] tested five potential devices, and consequently sites, of application of slow-release devices that could be used on cattle to identify the potential levels of dispersion of impregnated compounds through the normal behaviour of the cattle. The different sites and devices tested were head halters, ear tags, neck collars (bands), leg bands, and tail tags.

The study made use of a simple dispersion system with a coloured dye that was attached to one of the respective devices. The cattle were then observed for the consequent dispersal pattern from the dye, and conclusions were drawn on the potential distribution of acaricides from such devices. Each of the devices and locations tested demonstrated their own unique pattern of distribution of the dye, and the authors reinforced the notion that such devices should be matched to the target parasite [60]. The principle behind this is to ensure that the parasite in question is readily exposed to effective doses of the active compound in the device; especially when concentrations of these compounds will undoubtedly decrease after a period of time on the animal.

This is self-evident when one considers a prominent parasite such as the horn fly, *Haematobia irritans*, which has been the source of many different control strategies [61]. Through the behaviour of these flies surrounding the head, effective control has been achieved through the use of slow-release acaricide impregnated ear tags [61]. Similarly, for the brown ear tick, *Rhipicephalus appendiculatus,* for which acaricide-impregnated ear tags have been demonstrated as effective and are available [23]. Given the predilection site of this parasite, a slow-release tail tag would not be the most effective option and would be unlikely to provide meaningful levels of control.

When considering the predilection sites of *A. variegatum* and *A. hebraeum,* it is easy to understand why the researchers chose to make use of acaricide-impregnated tail tags to target these parasites. Observing the study of Beadles and colleagues it is seen that tail tags give the most widespread distribution of compound over the posterior end of cattle, and in turn to the bulk of the predilection sites preferred by these ticks [60].

The addition of an impregnated tag on the neck collar in these studies is further beneficial to help ensure that ticks attaching to the front end of the animal are also exposed to adequate concentrations of acaricidal compounds to achieve the lethal dose. However, understandably the use of an impregnated tag attached to the neck alone is unlikely to yield desired levels of control against these *Amblyomma* ticks.

It should be noted that these rudimentary distribution studies based on dyes did not include the effect of possible absorption and thus further distribution and enhanced efficacy of acaricidal compounds. In their study, Allan et al. [53] remarked that there was a good distribution of acaricidal compounds across all of the regions of the body that were tested within two days. This indicates the likelihood of effective and widespread distribution of compounds that is less dependent on physical transfer.

Distal limbs as a site for targeted tick control

From the studies conducted in West Africa on the dynamics and behaviour surrounding the infestation and attachment of cattle by *A. variegatum*, Stachurski suggested that a control method targeted at the feet of cattle may be effective in aiding control of these ticks [58, 62]. An acaricide footbath that could be used by cattle when returning from grazing in the late afternoon or evening was proposed.

This is particularly relevant to herds of cattle where the animals spend most of the day grazing in the field with very little time spent lying down, and are then brought into camps to rest in the evenings; as is seen in many communal farming systems across Africa. This is a targeted approach to kill ticks that are attached to the feet (primary attachment site) of cattle before they have the opportunity to migrate to the predilection sites on the cattle when the animals lie down.

The use of acaricide-based footbaths

An investigation into the efficacy of control achieved against *A. variegatum* through this method was conducted in West Africa [63]. The use of these routine footbaths containing various pyrethroids (alpha-cypermethrin, deltamethrin, and flumethrin) demonstrated good levels of control in reducing the levels of tick infestation on the predilection sites of these cattle. Regular dipping from the onset of the rainy season helped to prevent ticks from attaching to the predilection sites in as high numbers; however, it did not provide complete control in stopping ticks from infesting these sites. This was deemed to be beneficial as it would result in a level of control that would remain below the economic threshold but at the same time help animals maintain a level of endemic stability against diseases such as heartwater [63].

Following the data available on the pattern of infestation and attachment of *Amblyomma* ticks, it appears that a control measure centred on targeting the ticks on the legs of hosts is a viable option. In their studies in West Africa, through the use of acaricide footbaths for cattle, Stachurski and colleagues achieved notable results in controlling these ticks [63]. Such a targeted approach is beneficial for a number of reasons, but perhaps most prominent are the decreased costs and the ease of application of this method.

The use of impregnated leg bands

Furthermore, it was briefly mentioned that this two-stage infestation process of these ticks could suit the implementation of a control strategy making use of leg bands in cattle [58]. One could consider this proposed location in conjunction with the distribution studies that were conducted by Beadles et al. to help evaluate the possible efficacy of such an approach [60]. The distribution pattern of the substances used in their experiment indicate that there would be a good distribution of an acaricide to the lower limbs, inside surfaces of the legs, lower belly, dewlap and around the navel [60]. This accounts for a large proportion of the predilection sites of the *Amblyomma* ticks, with the exception of the neck, under the tail, and perineal region. However, one may be tempted to believe that the end of the tail would be likely to come into contact with such devices over a prolonged period which would then likely spread the agents further; but this is unsubstantiated at this time and would necessitate further testing.

Nonetheless, the distribution of these products could be of less importance if we return to the notion that a control measure that is targeted at the leg, such as a slow-release leg band, is based upon the two-stage infestation pattern of these ticks and targeting them at the primary attachment sites (lower limbs). Such a device would serve to expose the ticks to effective and lethal concentrations of the acaricidal compounds when they first attach to the lower limbs or feet while the animals are walking around and grazing. Thereby ticks would effectively be killed before they have the chance to migrate to their secondary and more permanent attachment sites on hosts.

The effect of such a slow-release leg band could further be potentiated through the addition of attraction-aggregation-attachment pheromone components along with an acaricide. Unfed adults of these *Amblyomma* ticks respond to the pheromones of feeding males to help them locate suitable sites of feeding; the adult females are in fact dependent on this signal and do not migrate to their secondary feeding sites without it.

It is thus believed that the addition of these AAAP components could help to prevent the redistribution of ticks to their secondary attachment sites, and rather cause them to aggregate or stay attached to the lower limbs of the hosts where these devices are applied. This would effectively help to increase the exposure time of these ticks to the active compounds of the acaricides but further reduce the damage that is caused by ticks when attaching to other sites on the host body such as the udder or scrotum.

Furthermore, it is believed that these decoy devices would greatly reduce the likelihood of ticks attaching directly to the predilection sites on animals. Therefore, this would help to mitigate the need for the acaricide to be well distributed in high concentrations across all regions of the body.

Conclusion

It is clear that while *Amblyomma* ticks have a significant impact on global health and production; they also provide the opportunity to explore new and targeted control methods against them. The influence that the attraction-aggregation-attachment pheromones have on their feeding behaviour and the infestation process of hosts is marked.

The studies illustrating the effects of these pheromonal components on ticks, further followed by the results of devices making use of these compounds for tick control are of great value. These studies, along with the findings in West Africa related to the infestation process of the tropical bont tick lay the foundation for the possibility of more targeted control measures against these ticks. It stands to reason that a technique that encompasses these two targeted approaches would yield promising results in the control of *Amblyomma* ticks.

Chapter 5: Conclusion and Recommendations

Literature supports the use of techniques and devices based upon the use of pheromones and other semiochemicals to augment the efficacy of more traditional control measures. The use of the semiochemical compounds promotes desired behaviour from these ticks; for instance, the AAAP compounds will encourage unfed adult ticks to move towards and aggregate around the source of the pheromone. By incorporating this into a trap or an “attract-and-kill” device such as the tags that have been discussed, it is possible to attain high levels of targeted control of ticks. This is then taken further when combining these techniques with knowledge of the biology and predilection site of the parasite to be controlled.

Existing devices attached to the distal limbs of livestock

The attachment and fixation of devices to the lower limbs of livestock is not a new concept. In fact, there are many different devices that are currently available and are in use in the cattle industry. Devices are attached to the lower limbs of cattle for identification purposes, but also increasingly as part of intelligent systems that are being used in the dairy industry (amongst others) to monitor production and bio-data of cattle on farms [64-66]. These devices come in a variety of designs but that most commonly seen is a band or strap that can be attached to the fetlock of the animals.

Empirical evidence suggests that this is a viable site of attachment for such a device as it can be fitted with ease when cattle are restrained, it does not interfere with normal behaviour or cause stress to the animal, it is easily visible, and when fitted correctly should remain attached to the animal with little difficulty.

This is in contrast to the impregnated tail tag decoys that have been tested as a control measure against *Amblyomma* ticks [53-55]. In these studies, the researchers encountered difficulties with the retention of these tail-tags for the duration of the full period of activity of the slow-release devices (three months). As has been discussed, the tail of cattle poses a difficult site on which to attach devices due to the great deal of stress and forces that a device in this location would experience with constant movement and flicking of the tail.

Further still, it is difficult to effectively attach a device to the tail without causing discomfort or ill-effects to the animal and tail. In their study into these devices, Norval and colleagues mentioned that there had been experiences of blood flow being cut off from the distal tail [52]. If it is fitted correctly, a device or band that is fitted to the lower limb does not present as many challenges in this regard and the risk of these undesirable effects is significantly less. Many of these devices are widespread, and it is possible to buy simple colour-coded plasticised bands commercially for cattle that can be used as part of an identification system (Fetlock Straps, KERBL).

The use of impregnated slow-release devices for control of ectoparasites

Impregnated slow-release devices may offer a viable and effective means of ectoparasite control. The use of these devices is not limited only to livestock or in fact to animals, and there are many examples of devices being used for human health. The use of plastics impregnated with insecticides such as pyrethroids to control mosquitoes and help with the control of malaria in humans is expanding [67].

This approach carries the benefit of being simple to implement on a wider scale and is able to provide enhanced levels of mosquito control for long-duration and is cost-effective. These are all very attractive qualities when considering the control of malaria and the fact that it is most prevalent in some of the poorest regions of the world. Slow-release devices are becoming widespread in small animal pets such as cats and dogs as well; with a flumethrin-imidacloprid-impregnated collar (Seresto® Bayer Animal Health) being widely available for purchase. In a comparative trial, these collars demonstrated excellent levels of control of fleas on cats for the full eight-month study [68].

Rechav [23] demonstrated the use of a slow-release acaricide-impregnated ear tag that could be used to control brown ear tick (*R. appendiculatus*) in cattle as early as the 1980s. The ear tags that demonstrated the best results were those impregnated with 7.5% flucythrinate, and when worn with one tag in each ear, they demonstrated 83% protection against brown ear tick for up to fifteen weeks. This has further been developed over the years and there is currently a commercially available product (CARAVANAS® Bupo Animal Health) containing 40% Diazinon in a slow-release, long-acting ear tag for cattle. The device is used as one ear tag per animal and is used to control horn fly *(H. irritans*) and brown ear tick (*R. appendiculatus*) for up to seventeen weeks.

The studies that were conducted in the Caribbean and Zimbabwe illustrated how effectively AAA pheromone compounds could be impregnated, as well as or along with acaricides, into PVC tags. These impregnated tags demonstrated significant results in the attraction and control of the *Amblyomma* ticks. However, furthermore they maintained effective levels of the active compounds for the duration that they were attached to animals (around three months in each study) [53-55].

There are a number of studies that have made use of slow-release acaricide-impregnated devices to varying extents and there a number of commercially available products. It is possible to impregnate a wide range of materials with a number of different chemical compounds such as insecticides or acaricides and pheromonal constituents. This facilitates the development of a wide range of products that can potentially be used to aid the control of external parasites.

Control of Amblyomma ticks through impregnated slow-release devices attached to distal limbs

Based on a review of the literature, a pheromone and acaricide-impregnated device that can be fitted to the lower limb or fetlock region of livestock for the control of *Amblyomma* ticks is proposed. This device could be manufactured by making use of designs from already-existing devices that are used for the identification of animals and impregnating these plastics with the desired compounds.

A plastic identification band (such as the fetlock bands sold by KERBL, to name one such device) would be well-suited for this purpose. Therefore, it would not be necessary to devise new attachment methods of such a device to overcome any issues that may have been faced in previous trials. The use of such a device as the impregnated tag would be favourable as these are made from durable materials and have been demonstrated to work on animals for sustained periods.

The plastic band could be impregnated with a combination of AAA pheromone constituents as has been previously discussed. The choice of a suitable acaricide to be impregnated should be based on several factors, but comprehensive efficacy studies and trials to compare the levels of control achieved from different compounds are recommended. Notwithstanding, previous trials indicate that some pyrethroids and synthetic pyrethroids such as deltamethrin and flumethrin demonstrate high levels of control against *Amblyomma* ticks when used in slow-release devices. It would further be beneficial to conduct comparative trials with pheromone-acaricide-impregnated leg bands to determine how many bands should be attached to a host in order to yield desired results.

It could be possible to achieve desired levels of tick control on an animal to be fitted with one of these devices through the use of just one band per animal. Alternatively, perhaps it would be more beneficial to attach them on alternating legs as in one band attached to the left foreleg and one right hindleg. This should be investigated further to help determine the feasibility and of this proposed control method and the levels of control that could be achieved.

It is recommended that a future comparative study be based on two prototypes; with "prototype A" being an acaricide-impregnated leg band, and "prototype B" being a leg band that is impregnated with the combination of pheromone components and an acaricide. These two prototypes or devices could be tested comparatively for the level of control achieved against *Amblyomma* species ticks; similarly to the trials conducted by Allan and colleagues in the Caribbean [53].

Benefits of AAAP-acaricide-impregnated leg bands

There are many benefits to the use of such a device to aid in the control of ticks. These slow-release devices are long-acting, and so they only need to be fitted infrequently (every three to eight months depending on the product). This is of great value as it helps to reduce management and labour costs associated with regular external parasite treatments, and further still helps provide a degree of assurance for farmers that their animals are protected against these parasites.

As has been mentioned, these devices are relatively easy and inexpensive to make and thus they offer a low-cost solution; and in fact, previous studies have shown them to be cheaper than the equivalent more traditional chemical control measures. Additionally, other resources will also be saved as this technique does not require the need for any water for application, and there is no need for specialised equipment.

If these devices are used correctly, and effective active acaricide compounds are used, they will help to provide high levels of control of a population of ticks. This would be dependent on a number of factors such as how many animals are fitted with tags and the extent of the range on which animals are allowed to travel. However, it is believed that even in areas with high tick burdens, these devices could have a significant impact in reducing and controlling the populations of *Amblyomma* ticks when used suitably and for a long enough time. Further studies will need to be undertaken to investigate these factors.

Additional benefits of making use of these impregnated leg bands is that they may offer flexibility on the number of leg bands that may be required and could be attached depending on the inherent tick burden, they could function additionally as a part of an identification system for animals (different groups have different colour tags), and they are simple for livestock owners to implement and control.

Pheromone-impregnated decoy devices that are attached to animals have an additional benefit over standalone devices or traps in that there is no need to make use of additional compounds and semiochemicals to replicate host factors, as these factors are already present. Therefore, the level of attraction and behavioural response from ticks is often higher for these devices used on animal hosts.

A further benefit of these proposed leg bands is that they are highly adaptable and can be used on different animals. There is no need to change the design to use them on cattle, goats or sheep, or even on different age groups, and the only difference that would be needed would be adapting the size of the bands. Different bands are already being used for different species of animals, and so it would be a simple adaptation of these devices as has been mentioned already.

Additional considerations and recommendations

Above some of the elements that have already been mentioned, it is recommended that some additional factors be investigated to determine the long-term feasibility and sustained use of these impregnated-devices as a widespread method of tick control. An important element to be investigated is that it should be evaluated what effect these devices would have on the control of other species of ticks also found in areas where these are to be implemented.

Although it is believed that the high levels of tick control that could be achieved through such a device may help reduce the development of acaricide resistance; and that it would, in fact, be more favourable than currently employed chemical control measures where sub-optimal dosing is common. Sub-optimal dosing can occur due to several reasons including incorrect weighing of animals, incorrect dose calculations and applications, or even through owners wishing to save costs by using less product. The use of these slow-release impregnated leg bands would help to avoid such issues as application, and use of these devices is very simplistic and clear, and less open to interpretation by owners.

It should further be investigated and seen as to whether high levels of organic materials such as mud or manure might affect these leg bands. Due to the high durability of these leg bands and their employed use in an expansive range of husbandry environments, it is believed that they will maintain their efficacy and function under these less-than-ideal circumstances. Nonetheless, it is a factor that deserves consideration and evaluation given that many dairy herds are reared in more temperate regions with high rainfall and consequently sodden pastures.

A final consideration related to the use of these impregnated leg bands in areas where various dangerous livestock diseases are endemic is the issue of development and maintenance of endemic resistance to these diseases in animal populations. If there is complete control of tick populations, then there will no longer be exposure of animals to ticks and the pathogens that they may carry, and thus this natural resistance or endemic stability will be lost in these populations of animals. However, if tick control is not absolute and there is still some exposure to ticks then this should not be a concern as ticks will be kept below the economic threshold but still maintain some immunity in these animals.

Conclusion

Amblyomma species ticks have been demonstrated as amongst the most significant ticks in the world through the diseases that they transmit and the direct effects that they have on health and production. This necessitates the need for novel and effective control strategies against these ticks. It is believed that a slow-release pheromone and acaricide-impregnated device that could be fitted to the legs of livestock could offer an effective tool for the control of *Amblyomma* species ticks.

This would offer a strategic control measure that is based upon the biology and behaviour of these ticks and is thus expected to yield levels of tick control equal or greater than traditional methods. This level of tick control can be achieved while using fewer resources than traditional methods and through an application method that is very simple to employ extensively. These proposed devices are believed to carry a wide range of potential benefits over traditional tick control measures, and it is believed that they demonstrate a need for increased investigation and further development.

Chapter 6: References

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Annexure

Ethics Approval

UNIVERSITEIT VAN PRETORIA
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Faculty of Veterinary Science

Research Ethics Committee

Project Title	**Pheromone-based tick control.**
Project Number	**REC060-19**
Researcher / Principal Investigator	**Dr SS van den Hurk**

Dlissertation / Thesis submitted for	**Masters**

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