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Acaricide resistance in livestock ticks infesting cattle in Africa: Current status and potential mitigation strategies

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A B S T R A C T

In many African countries, tick control has recently been the responsibility of resource-poor farmers rather than central government veterinary departments. This has led to an increase in acaricide resistance, threatening the welfare of livestock farmers in sub-Saharan Africa. Resistance has evolved to the three classes of acaricides used most extensively in the continent, namely fourth-generation synthetic pyrethroids (SP), organophosphates (OP) and amidines (AM), in virtually all countries in which they have been deployed across the globe. Most current data are derived from research in Australia and Latin America, with the majority of studies on acaricide resistance in Africa performed in South Africa. There is also limited recent research from West Africa and Uganda. These studies confirm that acaricide resistance in cattle ticks is a major problem in Africa. Resistance is most frequently directly assayed in ticks using the larval packet test (LPT) that is endorsed by FAO, but such tests require a specialist tick-rearing laboratory and are relatively time consuming. To date they have only been used on a limited scale in Africa and resistance is often still inferred from tick numbers on animals. Rapid tests for resistance in ticks, would be better than the LPT and are theoretically possible to develop. However, these are not yet available. Resistance can be mitigated through integrated control strategies, comprising a combination of methods, including acaricide class rotation or co-formulations, ethnoveterinary practices, vaccination against ticks and modified land management use by cattle, with the goal of minimising the number of acaricide applications required per year. There are data suggesting that small-scale farmers in Africa are often unaware of the chemical differences between different acaricide brands and use these products at concentrations other than those recommended by the manufacturers, or in incorrect rotations or combinations of the different classes of chemicals on the market. There is an urgent need for a more evidence-based approach to acaricide usage in small-scale livestock systems in Africa, including direct measurements of resistance levels, combined with better education of farmers regarding acaricide products and how they should be deployed for control of livestock ticks.

1. Introduction

Ticks are a major cause of economic loss in the livestock agricultural sector in the tropics and subtropics. The most important species of cattle ticks requiring control in Africa are *Rhipicephalus* (*Boophilus*) *decoloratus* and *Rhipicephalus* (*Boophilus*) *microplus*, which transmit the pathogens causing babesiosis (*Babesia bigemina* and *Babesia bovis*) and anaplasmosis (*Anaplasma marginale*); *Rhipicephalus appendiculatus* which transmits *Theileria parva*, the cause of East Coast fever; and several species in the genus *Amblyomma* (particularly *Am. variegatum*), which are responsible for transmission of *Ehrlichia ruminantium*, the cause of heartwater. The tick species infesting livestock in Africa have been comprehensively documented by Walker et al. (2003). Both *Rhipicephalus* spp. and *Amblyomma* spp. can also cause direct economic losses by infestation of

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cattle and other livestock, although this has never been quantified in Africa.

A range of chemicals have historically been used for control of ticks, but resistance has evolved to all of these, sometimes involving multiple resistance to different classes of chemicals (reviewed by George et al., 2004). The use of some of these acaricidal compounds, has been discontinued, for example organochlorines. The major classes of chemicals that are currently used in Africa are organophosphates (OP), fourth generation synthetic pyrethroids (SP) and amidines (AM). In some African countries, such as Tunisia for OP, use of certain compounds is prohibited. Much of the current literature on acaricide resistance derives from studies in Australia and South America, with the majority of African studies originating from South Africa. Thus, the available literature relating to acaricide resistance and mitigation strategies in sub-Saharan Africa is relatively limited. The first major class of modern acaricides were organochlorines, including dichlorodiphenyltrichloroethane (DDT), but these were withdrawn from the market due to their environmental persistence and accumulation along food chains through storage in body fat (Kuntz & Kemp, 1994). Organophosphate acaricides, which target the enzyme acetylcholinesterase, were introduced primarily to control organochlorine resistant *Rhipicephalus* (*Boophilus*) spp. ticks. However, resistance to OP has eliminated or decreased their usefulness in Australia, large parts of South America and also considerable areas of Africa (Kuntz & Kemp, 1994). Fourth generation cyano-substituted SP proved useful for control of organophosphate-resistant tick populations and were particularly effective when used in combination with OP (Schnitzerling et al., 1983). SP currently remain widely used in Africa; however, resistance to these compounds is increasingly widespread. AM (particularly amitraz) were demonstrated over a 5-year period in South Africa to be highly effective acaricides that were useful for control of all major economically important African ticks (Stanford et al., 1981), following initial successful trials in Australia and the USA. Two other distinct classes of chemicals that have been used as acaricides are macrocyclic lactones (ivermectin) and fipronil, a phenylpyrazole compound. However, these have been used on a limited scale in Africa, in case of the former due to cost, and the latter due to accumulation of residues in milk which prohibits application in the economically important dairy sector.

The initial deployment of acaricides involved formulations that could be diluted in water and applied to livestock through a hand sprayer, dipping tank or spray race. Hand spray delivery remains prominent in smallholder cattle systems in Africa; by contrast, dipping tanks have proved difficult to maintain, especially without government financial support, which is either minimal or not available in many countries. Additional delivery systems developed more recently include acaricide-impregnated ear-tags, intraruminal boluses, pour-on formulations and pheromone/acaricide-impregnated tail-tags. Although all of these methods show a degree of efficacy in trials, none has been widely adopted for routine application in Africa (Soll et al., 1990; Norval et al., 1996; Fourie et al., 2013; Kelley et al., 2014). The intraruminal bolus strategy that extends the duration of control relative to that from a single treatment was shown to deliver acaricide for 90 days with very efficient control of multiple tick species on calves in South Africa (Soll et al., 1990). However, it is likely to be too expensive for use on adult cattle. Ear-tags impregnated with a range of commonly used acaricides successfully controlled *R. appendiculatus* for up to 160 days (Young et al., 1985), but had minimal effect on other important tick species infesting the same herd. This factor, combined with the high costs, rendered this method unsuitable for general application. Pheromone/acaricide tail-tags were also too specific, because they were only effective in controlling *Amblyomma* spp. but not other co-infesting tick species.

Tick control using acaricides is ideally implemented as part of an integrated strategy that is tailored to the local tick ecology and the production goals of livestock keepers. Integrated control may include use of tick resistant cattle, strategic dipping with acaricide in response to economic thresholds as determined by modelling and switching pastures to avoid the build-up of tick populations. Combination with the TickGARD and Gavac® tick vaccines (Willadsen, 2004; de la Fuente & Contreras, 2015), which are based on a recombinant version of the BM86 gut protein, has also been used to minimise the frequency of chemical acaricide applications and the evolution of resistance. In some respects, these vaccines can be regarded as 'environmentally benign' acaricides, due to the requirement for regular re-vaccination to maintain adequate levels of immunity. To date vaccines have primarily been applied for control of *R. microplus* in Australia (TickGARD) and in Latin America (Gavac®) but have not yet been tested in more complex multi-species tick infestation scenarios in Africa.

2. Acaricide resistance in ticks

Resistance has rapidly evolved decreasing the effectiveness of all major classes of chemical acaricide tested for control of tick species infesting cattle (reviewed by Abbas et al., 2014; Dzemo et al., 2022). Given the remit of this review, we summarise only resistance to the acaricides that are currently widely used to control tick infestation of cattle in Africa. The history of development of resistance to acaricides has previously been reviewed in depth (George et al., 2004; Abbas et al., 2014). This article therefore presents only a brief summary of the data, and the reader is referred to these reviews for further information. Resistance to OP was recorded in multiple tick species, including *R. decoloratus*, *R. microplus*, *R. appendiculatus* and *Amblyomma variegatum*, between 1963 and 1986, in Australia, South American countries, Mexico, South Africa, Tanzania and Uganda. In the case of SP, resistance was first documented in 1978 with numerous subsequent reports in *R. microplus* (and also *R. decoloratus* in South Africa). Initial reports of resistance were from Australia, but other reports were subsequently documented in Brazil and Argentina, Mexico and South Africa. Recently, pyrethroid resistance has also been recorded in *R. microplus* in West Africa, following introduction of the tick from Brazil (Adehan et al., 2016). AM (amitraz) resistance was first demonstrated in Australia in 1981, followed by Brazil in 1995 and Mexico in 2002. Amitraz resistance has also been demonstrated more recently in South Africa (Mekonnen et al., 2003).

Apparent cases of resistance indicated by observation of the failure of chemical acaricides to effect control of tick populations in the field, require confirmation by *in vitro* bioassay testing in the laboratory to confirm diagnosis. Such confirmation is a prerequisite for developing objective resistance mitigation strategies. The most frequently used tests for assessing resistance to OP and pyrethroids, involving assessing tick mortality at a range of acaricide concentrations, are the larval packet test (LPT) and the larval immersion test (LIT) (reviewed by Kemp et al., 1998). The LPT has been recommended as a standard bioassay test by the Food and Agriculture Organisation of the United Nations (FAO, 1984) and is the most widely used method. However, although there are a few examples of the use of the LPT to confirm resistance of *R. microplus* to chemical acaricides in South Africa, West Africa and Uganda, *in vitro* resistance validation methods have not been regularly used when acaricide resistance is suspected in Africa. An adult immersion test (AIT) has also been developed (Sabatini et al., 2001). Additionally, a modified LPT involving the use of a nylon fibre substrate instead of filter paper is now available for confirmation of amitraz resistance in ticks (Miller et al., 2002). The LPT is not rapid to perform, it requires at least 35 days to obtain sufficient larvae of 7–14 days in age from one-host tick species such as *R. microplus* and potentially longer for multi-host ticks. A tick culture laboratory with trained personnel is also essential for performance of the LPT. Although the AIT is potentially quicker, in cases where the frequency of resistance is low, the difficulty of obtaining a sufficiently large number of engorged female ticks from untreated cattle limits its application (George et al., 2004). Where knowledge of acaricide resistance mechanisms is available at the molecular level, it would theoretically be desirable to develop more rapid tests based on PCR that could be applied to individual ticks (Abbas et al., 2014).

The molecular basis of acaricide resistance is known in at least some circumstances for several classes of compounds. OP are inhibitors of acetylcholinesterase (AChE), an enzyme vital to the function of the nervous system. Since 1950, ticks have developed resistance to more than 30 OPs and carbamates in 40 countries, with target-site insensitivity identified as the principal resistance mechanism (reviewed by Abbas et al., 2014). However, despite identification of at least six mutations in the AChE3 gene of an OP resistant *R. microplus* strain, these were not sufficient to confer OP resistance at the whole organism level, so additional mutations are likely to exist (Tameyer et al., 2013). Pyrethroids are neurotoxins that act on sodium ion channels and thus cause nerve excitation as a result of changes in nerve membrane permeabilities to sodium and potassium ions (Abbas et al., 2014). Target site mediated resistance was confirmed by Frank et al. (2013) who discovered a mutation in the Na+ ion channel that was subsequently shown to substantially decrease the channel sensitivity to pyrethroids (e.g. Vudriko et al., 2018a). However, evidence for a second mechanism of pyrethroid resistance in Mexican tick populations has also been demonstrated involving an esterase with permethrin-hydrolyzing activity. In the case of amitraz, which is an AM (amidine) compound, the mode of action is thought to be due its toxic effects on a receptor for the neuromodulator, octopamine. The molecular basis of target-site resistance appears to involve two nucleotide substitutions in the octopamine receptor in resistant strains of ticks that result in amino acid substitutions absent in all susceptible strains (Corley et al., 2013). In addition to these target site mutations, there is also evidence for the involvement of P450 monooxygenases (Abbas et al., 2014). These examples demonstrate that there are data on the molecular basis of resistance available for all three of the major classes of chemical acaricide that are frequently used in Africa. However, the resistance mechanisms described to date are from studies in Australia, South America and Mexico and not within Africa, so may not be directly applicable. These studies relate to *R. microplus*, a species which until relatively recently was not a serious problem in Africa, is now a serious emerging problem on the continent. Furthermore, as documented above, it has been shown that for all three classes of acaricide, AM, SP and OP, multiple resistance mechanisms can be present. Before PCR-based molecular tests can be usefully deployed in Africa, more research is required on resistance mechanisms in important indigenous African species, such as *R. appendiculatus* and *A. variegatum*, which may not be identical to those identified for *R. microplus*. A further important consideration is that any resistance mechanism forming the basis of a useful molecular test must be the predominant one present at control sites in the field.

3. Strategies for mitigation of acaricide resistance in ticks

Although acaricide resistance is likely to evolve in any tick control programme, and cannot be entirely prevented, the rate at which this occurs, will be variable according to a variety of factors, including the specific acaricides used and the frequency and modality of application. Additional factors include tick population genetics and dynamics, and life history strategies (Fig. 1). Anthropogenic factors such as underdosing and frequent tick treatment drive the selection and accumulation of resistant alleles in a tick population that are further dispersed by uncontrolled animal movement across boundaries in absence of quarantine measures. Changing climate is predicted to alter tick distribution especially in respect to cattle ticks that have higher propensity for accumulating acaricide resistance. Evidence-based information relating to the level of acaricide resistance in tick populations is potentially very important for identifying optimal tick control strategies, but such information, at the level of geographical resolution required, is seldom available even in well-resourced countries with serious livestock tick problems, such as Australia and the USA. A general principle in the design of tick control programmes is to keep the number of chemical acaricide treatments to a minimum, with the goal of delaying the development of resistance (George et al., 2004; Abbas et al., 2014). A number of strategies have been tested to attempt to achieve this goal.

Resistance monitoring, acaricide rotation and use of combinations of acaricides can help in preserving the efficacy of existing compounds. Regular monitoring is an essential part in delaying the development of resistance. Application of acaricides weekly, or every two weeks during the tick propagation season (typically the rainy season in tropical and subtropical Africa) is frequently employed in areas where tick resistance is prevalent. However, a high frequency of acaricide application is a risk factor for the emergence of resistant strains, is expensive and potentially negative for the environment, particularly through collateral damage to beneficial arthropod species. It has therefore been recommended by veterinarians that acaricide treatments should not exceed more than five per season (Abbas et al., 2014). Ideally cases of field resistance should be confirmed in the laboratory using a suitable *in vitro* assay such as LPT or AIT, and the data compared with existing management practices, but as already mentioned, although this has been implemented in a few projects in South Africa, Benin and Uganda, it is very far from standard practice across the continent.

Rotation of acaricides having different modes of action should theoretically reduce the selection pressure for resistance. Published accounts regarding the use of the acaricide rotation strategy to delaying the development of resistance in tick populations are few. However, one laboratory study showed that in an *R. microplus* strain subjected to deltamethrin, resistance was very high (resistance factor [RF] = 756) after 11 generations, whereas in the same *R. microplus* strain selected with deltamethrin followed by application of the organophosphate, coumaphos, in a rotational system, resistance to deltamethrin was very low (RF = 1.6) after 10 generations (Thullner et al., 2007). Rotation is not

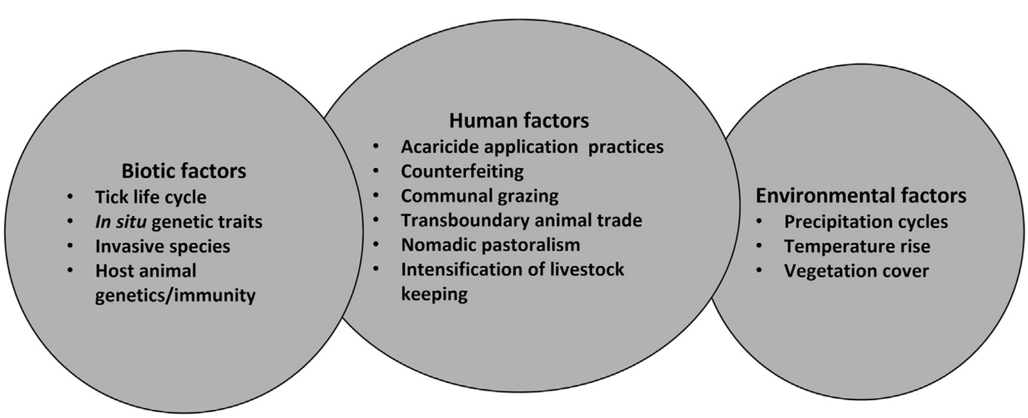

Fig. 1. Undercurrents driving the emergence of acaricide resistance in small-holding livestock systems in Africa. Similar to antimicrobial resistance, the convergence of human actions and environmental factors catalyses the selection of naturally occurring resistance traits in tick populations with anthropogenic actions driving the selection pressure, whereas suitable climatic conditions expedite emergence of resistant progenies as a result of shorter life-cycles and many tick generations per year.

easy to implement in practice on farm, and optimal practices regarding rotation strategy remain to be fully determined. For example, there are no clear guidelines regarding over what time-scale acaricides should be alternated.

The use of mixtures of acaricides is another potential approach to delay the emergence of resistance (Lovis et al., 2013). The rationale is based on the likelihood that one individual will not have resistant alleles to two chemicals with different modes of action. The synergistic effect of amitraz and permethrin against a permethrin-resistant *R. microplus* strain from Mexico has been evaluated (Fernández-Salas et al., 2012). Permethrin showed almost no mortality in the resistant strain even at the highest concentration but addition of amitraz to permethrin led to a dramatic increase in larval mortality. Constraints to this method are that acaricides must be of roughly equal persistence and compatible with respect to chemistry and formulation.

An alternative approach is the use of extracts from local indigenous plants for cheaper control of cattle ticks (Babar et al., 2012; Abbas et al., 2014). Because of the high cost of developing new acaricides, the application of botanicals to livestock in order to control the ectoparasites of veterinary importance is widespread particularly in the developing countries (Zaman et al., 2012). Twenty-one plants were identified with acaricidal activity in India (Abbas et al., 2014) and 13 plants having acaricidal properties have also been documented in Uganda (Robert et al., 2010). It is clear from the following examination of reports that there are many botanical products, derived from numerous plant families, that can kill ticks or inhibit oviposition (see Abbas et al., 2014). In some countries, commercially available plant-based formulations such as MyggA® 14 R.Z. Natural and Citriodiol® are being used for controlling ticks (Jaenson et al., 2006; Freitag & Kells, 2013). The integration of ethnoveterinary products with synthetic acaricides, seems not to have been fully explored and is certainly worth considering in an African context. A recent study by Arafa et al. (2020) exemplifies the potential of this strategy where the use of thyme and eucalyptus essential oils alongside deltamethrin against SP-resistant *Rhipicephalus* (*Boophilus*) *annulatus* resulted in synergistic deleterious effects targeting multiple resistance mechanisms, namely inhibition of acetylcholinesterase, increased lipid peroxidation, and oxidative stress. Therefore, the possibility of producing more cost-effective tick control products by combining extracts from local plants to decrease the quantities of chemical acaricides merits further research in an African context.

One additional supplementary control method, briefly mentioned earlier, is the deployment of tick vaccines. Commercially available vaccines for the control of *R. microplus* based on a recombinant form of a 'concealed' mid gut antigen known as BM86 are, TickGARD developed in Australia, and the similar Gavac® produced in Cuba (Willadsen et al., 1989; reviewed by de la Fuente et al., 2007). In the field, promising results have been obtained by using vaccines alone in Australia, Cuba and Mexico (de La Fuente et al., 1998, 2007). In Latin America, long-term use has resulted in reduction of acaricide application and lower prevalence of the major tick-borne pathogens *Babesia* spp. and *Anaplasma* spp. (reviewed by de la Fuente et al., 1998, 2007). Gavac® has also been tested in combination with acaricides and shown to reduce the amounts of acaricide required for control (Abbas et al., 2014). Homologues of the BM86 antigen are present across species of the genus *Rhipicephalus* and also in *Hyalomma* ticks and cross-protection using TickGARD against *R. annulatus* and *R. decoloratus* (Pipano et al., 2003; Odongo et al., 2007) has been demonstrated. The vaccine is theoretically most likely to be effective against one-host ticks, because of the cumulative effect of host immune response to the vaccine against all life-cycles present on the vaccinated animal. However, an effect of a recombinant version of the Ra86 homologue encoded by *R. appendiculatus*, a three-host tick, on moulting from larvae to adults has also been reported (Olds et al., 2012). By contrast, experimental vaccine trials carried out in Tunisia on *Hyalomma scupense* revealed that Bm86 has no effect on adults and juveniles of *H. scupense* as well as adults of *Hyalomma excavatum*, whilst the *H. scupense* orthologue, Hd86, was only effective against juveniles with an efficacy of 59.2% (Said et al., 2012). An important issue regarding the application of vaccination strategies in an African context is that *R. microplus*, an invasive Asian tick that is the vector of the globally most important livestock pathogens (*B. bovis* and *A. marginale*), has been present in South Africa for decades, and has more recently been identified in both Tanzania and Kenya in East Africa (Lynen et al., 2008; Kanduma et al., 2020). *Rhipicephalus microplus* has also recently been detected in western and central Africa, following two introductions of *R. microplus*-infested cattle from Brazil. This species appears to be rapidly displacing the indigenous African *R. decoloratus.* This has potential implications for the epidemiology of bovine babesiosis since most African cattle populations are naïve to *B. bovis*.

4. Tick acaricide resistance in Africa

Research in South Africa has demonstrated that indigenous *R. decoloratus* can develop resistance to the three major classes of acaricide that are currently deployed in Africa. The susceptibility of the larval offspring and engorged adult female *R. decoloratus*, collected from cattle on three dairy farms in the Eastern Cape Province, South Africa, was tested against the acaricides AM (amitraz), chlorfenvinphos and cypermethrin, by means of the LIT for larvae, and the reproductive estimate test (RET) and egg-laying test (ELT) for adults (Mekonnen et al., 2002). Although the results were to some degree variable between both the different farms and the specific tests used, assessment across the farms, detected resistance to all three categories of acaricide. Another study of *R. microplus* in a region of northern South Africa assessed acaricide resistance in combination with genetic diversity (Robbertse et al., 2016). The frequency of mutations potentially resulting in acaricide resistance was evaluated using single nucleotide polymorphisms (SNPs) in genes that contribute to acaricide insensitivity. A high prevalence of alleles potentially contributing to resistance against AM (amitraz) in the octopamine/tyramine (OCT/Tyr) receptor (frequency of 0.55) and to resistance to pyrethroids in the carboxylesterase-coding genes (frequency of 0.81) was observed. Following the recent introduction of *R. microplus*, acaricide resistance has already been detected in West Africa. An *in vitro* study was performed on five samples of *R. microplus* collected from five farms in four of the eight agro-ecological zones in Benin. The LPT was used to evaluate resistance to two SP (alpha cypermethrin and deltamethrin) and AM (amitraz) using a susceptible *Rhipicephalus geigyi* strain as a reference. Significant levels of resistance were detected on all except one farm (Adehan et al., 2016). In eastern and central Africa, the most detailed studies of acaricide resistance so far have been performed in Uganda (Vudriko et al., 2016, 2018b). Tick samples, primarily *R. appendiculatus* and *R. decoloratus*, were collected from 54 farms and the LPT was used to screen 31 tick populations for susceptibility to AM, SP, OP and organophosphate synthetic pyrethroid co-formulations (SOF). Resistance to SP was detected in 90.0% of the tick populations tested. Of serious concern, 60.0% and 63.0% of these ticks were 'super resistant' (exhibiting 0% mortality) against cypermethrin and deltamethrin, respectively. Resistance was also detected against SOF (43.3%), OP (chlorfenvinphos; 13.3%) and AM (amitraz; 12.9%). Multi-acaricide resistance was detected in 55.2% of resistant *Rhipicephalus* ticks and was significantly associated with use of both SP and COF to control *R. decoloratus*. Despite emergence of a degree of AM (amitraz) resistance in one region this was the most efficacious acaricide against SP- and COF-resistant *Rhipicephalus* (Vudriko et al., 2016). Recent studies in Tanzania (Nagagi et al., 2020) confirmed resistance of *R. microplus* and *Rhipicephalus evertsi* ticks in some districts to two commonly used SP (cypermethrin and deltamethrin), and also to the OP chlorfenviphos. It can be concluded that there is an increasing volume of evidence from multiple regions of the African continent, including, southern, western, central and eastern Africa for the existence of resistance to several classes of chemical acaricide. With the recent proliferation of the invasive Asian *R. microplus* in both western, central and eastern Africa where it is rapidly displacing the indigenous *R. decoloratus* (Lynen et al., 2008; Silatsa et al., 2019), this problem is likely to become more serious in future. *Rhipicephalus microplus* is known to rapidly acquire acaricide resistance and indeed, as mentioned above, introduced populations from Brazil may already be resistant.

In North Africa where a seasonal climate with cold winters occurs, several major cattle ticks of the genus *Hyalomma* produce a single tick generation per year resulting in a more moderate cattle infestation quantum comparatively to tropical African regions. Consequently, risks of acaricide resistance selection are lower given the reduced need for frequent acaricide application and the lower rate of tick population growth. Nevertheless, a recent study (El Hachimi et al., 2022) provides an indication that resistance is either present or emerging for diazinon and AM (amitraz) in Morocco for the cattle tick *Hyalomma marginatum*. Also, similar studies in Egypt have documented *R. annulatus* resistance to ivermectin (El Ashram et al., 2019) and deltamethrin (Arafa et al., 2021).

5. Acaricide use and potential resistance mitigation strategies in African smallholder systems

There are several recent studies of acaricide deployment by farmers in East Africa including Uganda (Vudriko et al., 2016, 2018b), Tanzania (Nagaki et al., 2020) and Kenya (Kamidi & Kamidi, 2005; Mutavi et al., 2021). Typically, acaricide application in these systems is by use of hand sprayers that are either individually, or communally, owned by livestock farmers. A common feature is that small-scale farmers are poorly educated about the nature and use of these products and that the concentrations used after dilution are frequently not those recommended by the manufacturers. A recent study in Laikipia central Kenya revealed serious misuse of acaricides in a predominantly AM (amitraz-based) acaricidal regime (Mutavi et al., 2021). This can lead to underdosing and selection for resistance. The time-scales of application are often weekly or sometimes twice weekly, but it is far from clear whether this is optimal. In addition, unsuitable combinations of acaricides (sometimes also mixed with other classes of pesticides) or suboptimal timing of rotation of the different classes of acaricide is prevalent in smallholder livestock production systems. There is therefore a need for better farmer education regarding the chemicals present in acaricides, through the agricultural extension system and community networks, and also improved scrutiny by the government regulatory bodies (Fig. 2). As mentioned previously, AM (amitraz) tends to be the most frequently used acaricide by African smallholders, although OP and SP are also widely deployed. The preference for AM (amitraz) is logical since amidines appear to have the lowest levels of resistance where this has been measured by assays such as the LPT, for example in Uganda (Vudriko et al., 2016). By contrast, studies frequently reveal high levels of resistance to the commonly used SPs, deltamethrin and cypermethrin, questioning the long-term efficacy of these products as a stand-alone control measure.

One African tick genus that does not seem to exhibit frequent resistance is *Amblyomma*, although resistance in *Amblyomma hebraeum* has been observed in South Africa (Mekonnen et al., 2002). In semi-arid areas of West Africa where *Am. variegatum* is the most important tick species, it transmits *Ehrlichia ruminantium* (the cause of heartwater). This results in considerable direct damage to livestock. In these West African agro-ecosytems acaricide application is mainly implemented using

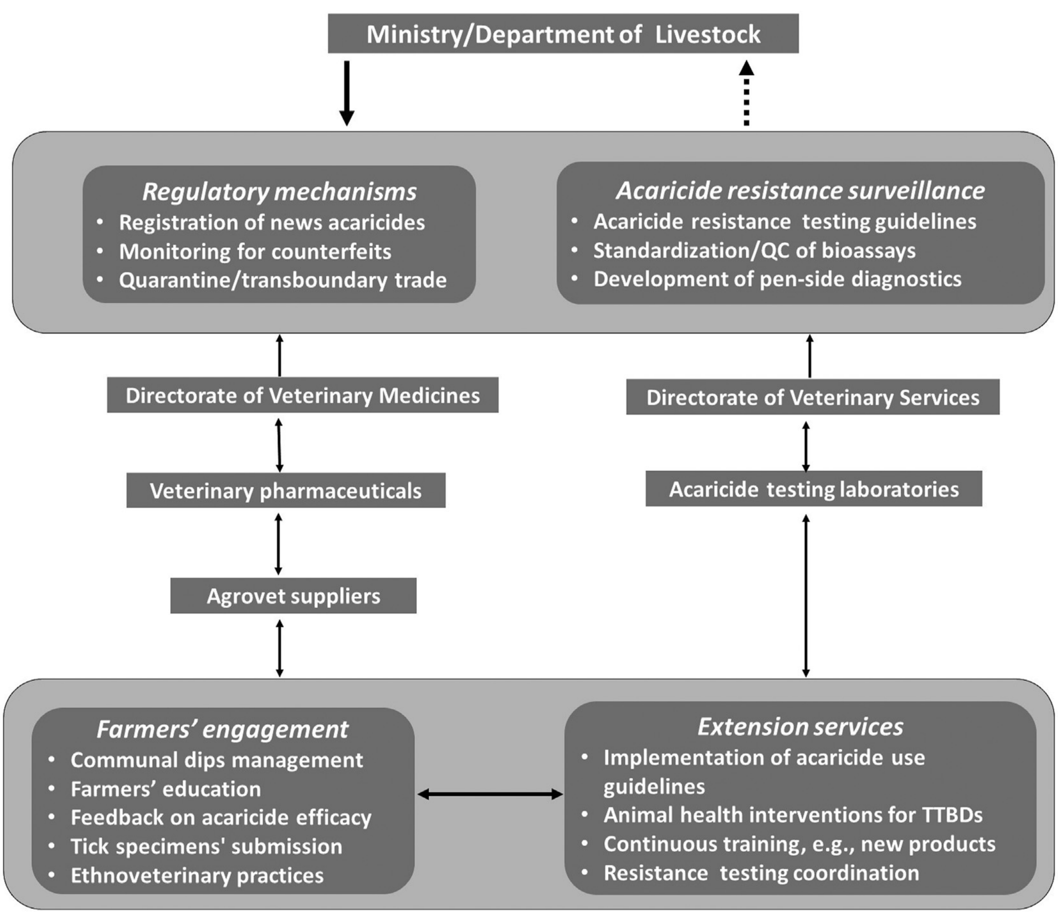

Fig. 2. A policy framework for acaricide resistance mitigation in low-income countries. The proximity of extension services to the farmers is a strategic point for intervention in mitigating acaricide resistance in settings such as those found in Africa. Farmers' education coupled with strengthening of last mile veterinary services are critical components for a successful strategy to reverse or delay the establishment of acaricide resistance. A national or regional network of resistance testing laboratories can provide critical epidemiological data on the patterns and intensity of resistance by tick species, acaricidal compounds and region to underpin rational intervention measures.

portable manual sprayers or direct physical removal of ticks (Adehan et al., 2018). An alternative delivery strategy that has been tested in Burkina Faso is the use of foot-baths, rather than spraying (de Meneghie et al., 2016). This is more economical, since it uses less acaricide and is effective because *Am. variegatum* ticks infest cattle *via* the hooves and legs.

There have been few studies of the effectiveness of different acaricidal application regimes in Africa. One interesting investigation is a long-term longitudinal study in western Kenya which demonstrated that switching from AM (amitraz) to an organophosphate acaricide, when the local *Rhipicephalus* tick populations had become resistant to the former was not effective (Kamidi & Kamidi, 2005). However, when a 'relay' system utilising first amitraz and then an OP at a 3-day interval, was adopted, minimal resistance was observed over three subsequent years. There have also been relatively few studies in smallholder systems in which resistance was directly measured using assays such as the LPT (or modified LPT in the case of amitraz). A benchmark study in East Africa is that of Vudriko and colleagues in Uganda (Vudriko et al., 2016). These authors have also suggested a strategy for evidence-based management of acaricide resistance that involves farmers reporting resistance and submitting tick samples to a central tick laboratory which performs bioassays to ascertain resistance levels. A dialogue involving manufacturers, veterinary services and other stakeholders then ultimately leads to the creation of objective policy recommendations for acaricide management by farmers (Vudriko et al., 2018b). This represents a productive strategy for enhanced future tick control and could serve as a template for future research and policy recommendations (Fig. 2).

6. Future directions

It is evident that acaricide resistance represents a rapidly growing problem to livestock production in the African continent. The dispersal of *R. microplus*, and intensification of the dairy sector through use of exotic taurine breeds or crossbreeds are among factors that will result in increased acaricide resistance in the region. Successful mitigation strategies underpinned by accurate data on tick resistance and relevant government policies, are therefore urgently needed to protect African livestock and enhance the livelihoods of those dependent on animal farming. Whereas a wide range of specific interventions have been prescribed (e.g. Abbas et al., 2014), some of these may not be feasible or practical in the context of small-scale livestock systems in Africa. In our assessment, the following measures could have fast and direct impact on tick control and ultimately, reduce acaricide resistance in the region.

- Farmers' education: In many parts of Africa, tick control is the responsibility of individual farmers; there is therefore a clear need to educate farmers on the right approach to the sustainable acaricide use including optimum dosing levels and frequency of treatment. It will also be important to provide knowledge of methodologies allowing monitoring whether resistance is present.
- Acaricide resistance monitoring at national or regional level, and harmonisation of vector control policies, e.g. border quarantine and regulation of acaricide registration and marketing. Particularly important is also the need to train a critical mass of personnel from the region on the LPT to provide standardized data on the status of tick resistance in the field.
- Better animal nutrition to improve host animals' immunity to tick infestation: Host resistance to ticks represents an economical and scalable approach to reduce tick infestation (Frisch, 1999). This innate resistance is mostly underpinned by the cattle's immunity system, which in turn becomes severely impaired during periods of nutritional deficiency (Maryam et al., 2012; Mattioli et al., 2020). Interventions that improve feed quality and availability will decrease tick density on animals and hence the need for frequent acaricide treatment.
- Anti-tick vaccine deployment: Due to the presence of multiple important tick genera in much of tropical Africa, vaccination with current tick vaccines is unlikely to provide a stand-alone solution. Combining vaccination with modified thresholds for initiating acaricide treatment can augment the economic benefits of integrated tick control strategy especially against the *Rhipicephalus* (*Boophilus*) spp.
- Continuous research on the development and validation of molecular assays for acaricide resistance to complement the LPT.

7. Conclusions

Intensive application of chemical acaricides remains the mainstay of vector control globally. Therefore, the need for preserving the efficacy and longevity of existing acaricides cannot be overstated. Worryingly, new patterns of cattle tick distribution in the African continent are emerging against a backdrop of increased replacement of indigenous resistant cattle with the high yielding, but susceptible exotic taurine breeds. Tick resistance threatens food and nutritional security especially in Africa where the consumption of animal source foods (ASF) is inadequate although currently rising (Jabbar et al., 2010). Intervention measures need to be multi-pronged and sustained, and the magnitude of the challenge spelt out clearly to stakeholders. Key research gaps such as elucidating the social and economic drivers of acaricide use among smallholder livestock keepers need to be addressed with the ultimate goal of optimising interventions in specific small-scale livestock systems.

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CRediT author statement

NG, EK, BW and RB: conceived the project. NG and RB: writing - original draft. NG, EK, BW, MD and RB: writing - review & editing. All authors read and approved the final manuscript.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abbas, R.Z., Zaman, M.A., Colwell, D.D., Gilleard, J., Iqbal, Z., 2014. Acaricide resistance in cattle ticks and approaches to its management: the state of play. Vet. Parasitol. 203, 6–20. https://doi.org/10.1016/j.vetpar.2014.03.006.

Adehan, S.B., Adakal, H., Gbinwoua, D., Yokossi, D., Zoungrana, S., Toé, P., et al., 2018. West African cattle farmers' perception of tick-borne diseases. EcoHealth 15, 437–449. https://doi.org/10.1007/s10393-018-1323-8.

Adehan, S.B., Biguezoton, A., Adakal, H., Assogba, M.N., Gbaguidi, A.M., Tonouhewa, A., 2016. Acaricide resistance of *Rhipicephalus microplus* ticks in Benin. Afr. J. Agric. Res. 11, 1199–1208. https://doi.org/10.5897/AJAR2015.10619.

Arafa, W.M., Aboelhadid, S.M., Moawad, A., Shokeir, K.M., Ahmed, O., de León, A.A.P., 2021. Control of *Rhipicephalus annulatus* resistant to deltamethrin by spraying infested cattle with synergistic eucalyptus essential oil-thymol-deltamethrin combination. Vet. Parasitol. 290, 109346. https://doi.org/10.1016/j.vetpar.2021.109346.

Arafa, W.M., Klafke, G.M., Tidwell, J.P., de León, A.A.P., Esteve-Gassent, M., 2020. Detection of single nucleotide polymorphism in the para-sodium channel gene of *Rhipicephalus annulatus* populations from Egypt resistant to deltamethrin. Ticks Tick Borne Dis. 11, 101488. https://doi.org/10.1016/j.ttbdis.2020.101488.

Babar, W., Iqbal, Z., Khan, M.N., Muhammad, G., 2012. An inventory of the plants used for parasitic ailments of animals. Pakistan Vet. J. 32, 183–187.

Corley, S.W., Jonsson, N.N., Piper, E.K., Cutullé, C., Stear, M.J., Seddon, J.M., 2013. Mutation in the RmβAOR gene is associated with amitraz resistance in the cattle tick *Rhipicephalus microplus*. Proc. Natl. Acad. Sci. USA 110, 16772–16777. https://doi.org/10.1073/pnas.1309072110.

De la Fuente, J., Contreras, M., 2015. Tick vaccines: current status and future directions. Expert Rev. Vaccines 14, 1367–1376. https://doi.org/10.1586/14760584.2015.1076339.

De la Fuente, J., Almazán, C., Canales, M., Pérez de la Lastra, J.M., Kocan, K.M., Willadsen, P., 2007. A ten-year review of commercial vaccine performance for control of tick infestations on cattle. Anim. Health Res. Rev. 8, 23–28. https://doi.org/10.1017/S1466252307001193.

De La Fuente, J., Rodríguez, M., Redondo, M., Montero, C., García-García, J., Méndez, L., et al., 1998. Field studies and cost-effectiveness analysis of vaccination with Gavac against the cattle tick *Boophilus microplus*. Vaccine 16, 366–373. https://doi.org/10.1016/S0264-410X(97)00208-9.

De Meneghi, D., Stachurski, F., Adakal, H., 2016. Experiences in tick control by acaricide in the traditional cattle sector in Zambia and Burkina Faso: possible environmental and public health implications. Front. Publ. Health 4, 239. https://doi.org/10.3389/fpubh.2016.00239.

Dzemo, W.D., Thekisoe, O., Vudriko, P., 2022. Development of acaricide resistance in tick populations of cattle: a systematic review and meta-analysis. Heliyon 8, 08718. https://doi.org/10.1016/J.HELIYON.2022.E08718.

El-Ashram, S., Aboelhadid, S.M., Kamel, A.A., Mahrous, L.N., Fahmy, M.M., 2019. First report of cattle tick *Rhipicephalus* (*Boophilus*) *annulatus* in Egypt resistant to ivermectin. Insects 10, 404. https://doi.org/10.3390/insects10110404.

Elhachimi, L., Van Leeuwen, T., Dermauw, W., Rogiers, C., Valcárcel, F., Olmeda, A.S., et al., 2022. Variation of diazinon and amitraz susceptibility of *Hyalomma marginatum* (Acari: Ixodidae) in the Rabat-Sale-Kenitra region of Morocco. Ticks Tick borne Dis. 13, 101883. https://doi.org/10.1016/j.ttbdis.2021.101883.

FAO, 1984. Ticks and Tick Borne Disease Control. A Practical Field Manual, Vol. I. In: Tick Control. Food and Agriculture Organisation of the United Nations, Rome.

Fernández-Salas, A., Rodríguez-Vivas, R.I., Alonso-Díaz, M.Á., 2012. Resistance of *Rhipicephalus microplus* to amitraz and cypermethrin in tropical cattle farms in Veracruz, Mexico. J. Parasitol. 98, 1010–1014. https://doi.org/10.1645/GE-3074.1.

Fourie, J.J., Liebenberg, J.E., Nyangiwe, N., Austin, C., Horak, I.G., Bhushan, C., 2013. The effects of a pour-on formulation of fluazuron 2.5% and flumethrin 1% on populations of *Rhipicephalus decoloratus* and *Rhipicephalus microplus* both on and off bovine (Bosmara breed) hosts. Parasitol. Res. 112 (Suppl. 1), 67–69. https://doi.org/10.1007/S00436-013-3282-X.

Freitag, J.A., Kells, S.A., 2013. Efficacy and application considerations of selected residual acaricides against the mold mite *Tyrophagus putrescentiae* (Acari: Acaridae) in simulated retail habitats. J. Econ. Entomol. 106, 1920–1926. https://doi.org/10.1603/EC13038.

Frisch, J.E., 1999. Towards a permanent solution for controlling cattle ticks. Int. J. Parasitol. 29, 57–71. https://doi.org/10.1016/S0020-7519(98)00177-5.

George, J.E., Pound, J.M., Davey, R.B., 2004. Chemical control of ticks on cattle and the resistance of these parasites to acaricides. Parasitology 129 (Suppl. 1), 353–366. https://doi.org/10.1017/S0031182003004682.

Jabbar, M.A., Baker, D., Fadiga, M.L. (Eds.), 2010. Demand for Livestock Products in Developing Countries with a Focus on Quality and Safety Attributes: Evidence from Asia and Africa, vol. 24. ILRI, Nairobi, Kenya. ILRI Research Report.

Jaenson, T.G., Garboui, S., Pålsson, K., 2006. Repellency of oils of lemon eucalyptus, geranium, and lavender and the mosquito repellent MyggA natural to *Ixodes ricinus* (Acari: Ixodidae) in the laboratory and field. J. Med. Entomol. 43, 731–736. https://doi.org/10.1093/jmedent/43.4.731.

Kamidi, R.E., Kamidi, M.K., 2005. Effects of a novel pesticide resistance management strategy on tick control in a smallholding exotic-breed dairy herd in Kenya. Trop. Anim. Health Prod. 37, 469–478. https://doi.org/10.1007/S11250-005-2173-2.

Kanduma, E.G., Emery, D., Githaka, N.W., Nguu, E.K., Bishop, R.P., Šlapeta, J., 2020. Molecular evidence confirms occurrence of *Rhipicephalus microplus* Clade A in Kenya and sub-Saharan Africa. Parasit. Vectors 13, 432. https://doi.org/10.1186/S13071-020-04266-0.

Kelly, P.J., Lucas, H.M., Randolph, C.M., Ackerson, K., Blackburn, J.K., Dark, M.J., 2014. Efficacy of slow-release tags impregnated with aggregation-attachment pheromone and deltamethrin for control of *Amblyomma variegatum* on St. Kitts, West Indies. Parasit. Vectors 7, 182. https://doi.org/10.1186/1756-3305-7-182.

Kemp, D.H., 1998. Acaricide resistance in the cattle ticks *Boophilus microplus* and *Boophilus decoloratus*: Review of resistance data; Standardisation of resistance tests and recommendations for integrated parasite control to delay resistance. Food and Agriculture Organisation of the United Nations, Rome. Report to the Animal Health Services, pp. 1-32. http://hdl.handle.net/102.100.100/217076.

Kunz, S.E., Kemp, D.H., 1994. Insecticides and acaricides: Resistance and environmental impact. Rev. Sci. Tech. 13, 1249–1286. https://doi.org/10.20506/RST.13.4.816.

Lovis, L., Reggi, J., Berggoetz, M., Betschart, B., Sager, H., 2013. Determination of acaricide resistance in *Rhipicephalus* (*Boophilus*) *microplus* (Acari: Ixodidae) field populations of Argentina, South Africa, and Australia with the larval tarsal test. J. Med. Entomol. 50, 326–335. https://doi.org/10.1603/ME12127.

Lynen, G., Zeman, P., Bakuname, C., Di Giulio, G., Mtui, P., Sanka, P., Jongejan, F., 2008. Shifts in the distributional ranges of *Boophilus* ticks in Tanzania: evidence that a parapatric boundary between *Boophilus microplus* and *B. decoloratus* follows climate gradients. Exp. Appl. Acarol. 42, 147–164. https://doi.org/10.1007/S10493-008-9134-1.

Maryam, J., Babar, M.E., Nadeem, A., Hussain, T., 2012. Genetic variants in interferon gamma (IFN-γ) gene are associated with resistance against ticks in *Bos taurus* and *Bos indicus*. Mol. Biol. Rep. 39, 4565–4570. https://doi.org/10.1007/s11033-011-1246-8.

Mattioli, G.A., Rosa, D.E., Turic, E., Picco, S.J., Raggio, S.J., Minervino, A.H.H., et al., 2020. Effects of parenteral supplementation with minerals and vitamins on oxidative stress and humoral immune response of weaning calves. Animals 10, 1298. https://doi.org/10.3390/ani10081298.

Mekonnen, S., Bryson, N.R., Fourie, L.J., Peter, R.J., Spickett, A.M., Taylor, R.J., et al., 2003. Comparison of 3 tests to detect acaricide resistance in *Boophilus decoloratus* on dairy farms in the Eastern Cape Province, South Africa. J. S. Afr. Vet. Assoc. 74, 41–44. https://hdl.handle.net/10520/EJC99523.

Mekonnen, S., Bryson, N.R., Fourie, L.J., Peter, R.J., Spickett, A.M., Taylor, R.J., et al., 2002. Acaricide resistance profiles of single-and multi-host ticks from communal and commercial farming areas in the Eastern Cape and North-West Provinces of South Africa. Onderstepoort J. Vet. Res. 69, 99–105. http://hdl.handle.net/2263/18296.

Miller, R.J., Davey, R.B., George, J.E., 2002. Modification of the food and agriculture organization larval packet test to measure amitraz-susceptibility against Ixodidae. J. Med. Entomol. 39, 645–651. https://doi.org/10.1603/0022-2585-39.4.645.

Mutavi, F., Heitkönig, I., Wieland, B., Aarts, N., Van Paassen, A., 2021. Tick treatment practices in the field: Access to, knowledge about, and on-farm use of acaricides in Laikipia, Kenya. Ticks Tick Borne Dis. 12, 101757. https://doi.org/10.1016/j.ttbdis.2021.101757.

Nagagi, Y.P., Kimaro, E.G., Temba, V., 2020. Practical application and the possible emergence of tick resistance to commonly used acaricides in various districts of Tanzania. Livestock Res. Rural Devel. 32, 8. http://lrrd.cipav.org.co/lrrd32/8/petnag32127.html.

Norval, R.A.I., Sonenshine, D.E., Allan, S.A., Burridge, M.J., 1996. Efficacy of pheromone-acaricide-impregnated tail-tag decoys for controlling the bont tick, *Amblyomma hebraeum* (Acari: Ixodidae), on cattle in Zimbabwe. Exp. Appl. Acarol. 20, 31–46. https://doi.org/10.1007/BF00051475.

Odongo, D., Kamau, L., Skilton, R., Mwaura, S., Nitsch, C., Musoke, A., et al., 2007. Vaccination of cattle with TickGARD induces cross-reactive antibodies binding to conserved linear peptides of Bm86 homologues in *Boophilus decoloratus*. Vaccine 25, 1287–1296. https://doi.org/10.1016/J.VACCINE.2006.09.085.

Olds, C., Mwaura, S., Crowder, D., Odongo, D., Van Oers, M., Owen, J., et al., 2012. Immunization of cattle with Ra86 impedes *Rhipicephalus appendiculatus* nymphal-to-adult molting. Ticks Tick Borne Dis. 3, 170–178. https://doi.org/10.1016/j.ttbdis.2012.03.003.

Opiro, R., Akol, A.M., Okello-Onen, J., 2010. Ethnoveterinary botanicals used for tick control in the Acholi subregion of Uganda. J. Anim. Vet. Adv. 9, 2951–2954. https://doi.org/10.3923/javaa.2010.2951.2954.

Pipano, E., Alekceev, E., Galker, F., Fish, L., Samish, M., Shkap, V., 2003. Immunity against *Boophilus annulatus* induced by the Bm86 (Tick-GARD) vaccine. Exp. Appl. Acarol. 29, 141–149. https://doi.org/10.1023/A:1024246903197.

Rinkevich, F.D., Du, Y., Dong, K., 2013. Diversity and convergence of sodium channel mutations involved in resistance to pyrethroids. Pestic. Biochem. Physiol. 106, 93–100. https://doi.org/10.1016/J.PESTBP.2013.02.007.

Robbertse, L., Baron, S., van der Merwe, N.A., Madder, M., Stoltsz, W.H., Maritz-Olivier, C., 2016. Genetic diversity, acaricide resistance status and evolutionary potential of a *Rhipicephalus microplus* population from a disease-controlled cattle farming area in South Africa. Ticks Tick Borne Dis. 7, 595–603. https://doi.org/10.1016/J.TTBDIS.2016.02.018.

Sabatini, G.A., Kemp, D.H., Hughes, S., Nari, A., Hansen, J., 2001. Tests to determine LC50 and discriminating doses for macrocyclic lactones against the cattle tick, *Boophilus microplus*. Vet. Parasitol. 95, 53–62. https://doi.org/10.1016/S0304-4017(00)00406-4.

Said, M.B., Galai, Y., Canales, M., Nijhof, A.M., Mhadhbi, M., Jedidi, M., et al., 2012. Hd86, the Bm86 tick protein ortholog in *Hyalomma scupense* (syn. *H. detritum*): expression in *Pichia pastoris* and analysis of nucleotides and amino acids sequences variations prior to vaccination trials. Vet. Parasitol. 183, 215–223. https://doi.org/10.1016/j.vetpar.2011.07.049.

Schnitzerling, H.J., Nolan, J., Hughes, S., 1983. Toxicology and metabolism of some synthetic pyrethroids in larvae of susceptible and resistant strains of the cattle tick *Boophilus microplus* (Can.). Pestic. Sci. 14, 64–72.

Silatsa, B.A., Kuiate, J.R., Njiokou, F., Simo, G., Feussom, J.M.K., Tunrayo, A., et al., 2019. A countrywide molecular survey leads to a seminal identification of the invasive cattle tick *Rhipicephalus* (*Boophilus*) *microplus* in Cameroon, a decade after it was reported in Côte d'Ivoire. Ticks Tick Borne Dis. 10, 585–593. https://doi.org/10.1016/J.TTBDIS.2019.02.002.

Soll, M.D., Benz, G.W., Carmichael, I.H., Gross, S.J., 1990. Efficacy of ivermectin delivered from an intraruminal sustained-release bolus against natural infestations of five African tick species on cattle. Vet. Parasitol. 37, 285–296. https://doi.org/10.1016/0304-4017(90)90011-Y.

Stanford, G.D., Baker, J.A.F., Ratley, C.V., Taylor, R.J., 1981. The development of a stabilized amitraz cattle dip for control of single and multi-host ticks and their resistant strains in South Africa. In: Whitehead, G.B., Gibson, J.D. (Eds.), Tick Biology and Control: Proceedings of an International Conference, January 27-29, 1981. Tick Research Unit, Rhodes University, Grahamstown, South Africa, pp. 143–181.

Temeyer, K.B., Tuckow, A.P., Brake, D.K., Li, A.Y., Perez De León, A.A., 2013. Acetylcholinesterases of blood-feeding flies and ticks. Chem. Biol. Interact. 203, 319–322. https://doi.org/10.1016/J.CBI.2012.09.010.

Thullner, F., Willadsen, P., Kemp, D., 2007. Acaricide rotation strategy for managing resistance in the tick *Rhipicephalus* (*Boophilus*) *microplus* (Acarina: Ixodidae):

laboratory experiment with a field strain from Costa Rica. J. Med. Entomol. 44, 817–821. https://doi.org/10.1093/JMEDENT/44.5.817.

Vudriko, P., Okwee-Acai, J., Byaruhanga, J., Tayebwa, D.S., Omara, R., Muhindo, J.B., et al., 2018a. Evidence-based tick acaricide resistance intervention strategy in Uganda: concept and feedback of farmers and stakeholders. Ticks Tick Borne Dis. 9, 254–265. https://doi.org/10.1016/J.TTBDIS.2017.09.011.

Vudriko, P., Okwee-Acai, J., Tayebwa, D.S., Byaruhanga, J., Kakooza, S., Wampande, E., et al., 2016. Emergence of multi-acaricide resistant *Rhipicephalus* ticks and its implication on chemical tick control in Uganda. Parasit. Vectors 9, 4. https://doi.org/10.1186/s13071-015-1278-3.

Vudriko, P., Umemiya-Shirafuji, R., Okwee-Acai, J., Tayebwa, D.S., Byaruhanga, J., Bbira, J.S., et al., 2018b. C190A knockdown mutation in sodium channel domain II of pyrethroid-resistant *Rhipicephalus appendiculatus*. Ticks Tick Borne Dis. 9, 1590–1593. https://doi.org/10.1016/J.TTBDIS.2018.08.007.

Walker, A.R., Bouattour, A., Camicas, J.-L., Estrada-Peña, A., Horak, I.G., Latif, A.A., et al., 2003. Ticks of Domestic Animals in Africa: A Guide to Identification of Species. Atlanta, Houten, the Netherlands.

Willadsen, P., 2004. Anti-tick vaccines. Parasitology 129 (Suppl. 1), S367–S387. https://doi.org/10.1017/S0031182003004657.

Willadsen, P., Riding, G.A., McKenna, R.V., Kemp, D.H., Tellam, R.L., Nielsen, J.N., et al., 1989. Immunologic control of a parasitic arthropod. Identification of a protective antigen from *Boophilus microplus*. J. Immunol. 143, 1346–1351.

Young, A.s., De Castro, J.J., Kiza-Auru, P.P., 1985. Control of tick (Acari: Ixodidae) infestation by application of ear tags impregnated with acaricides to cattle in Africa. Bull. Entomol. Res. 75, 609–619. https://doi.org/10.1017/S0007485300015868.

Zaman, M.A., Iqbal, Z., Abbas, R.Z., Khan, M.N., Muhammad, G., Younus, M., Ahmed, S., 2012. *In vitro* and *in vivo* acaricidal activity of a herbal extract. Vet. Parasitol. 186, 431–436. https://doi.org/10.1016/j.vetpar.2011.11.018.