THE DIAGNOSIS AND MITIGATION OF ACARICIDE RESISTANCE IN POPULATIONS OF TICKS AFFECTING CATTLE

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Abstract

Resistance of ticks to acaricides is a major problem for cattle producers in subtropical and tropical areas where ticks, especially *Boophilus microplus*, and the disease agents they transmit are a constraint to cost-effective production. Few acaricides are being developed and marketed as replacements for chemicals rendered ineffective by the evolution of resistance, and new products are often significantly more expensive. New and improved approaches to the diagnosis and mitigation of resistance of ticks to acaricides are needed to extend the useful life of both existing and new compounds. Reliable diagnostic tools are required for determining that resistance is responsible for tick control failures, selecting an alternative product when resistance is diagnosed, and research to develop tick control strategies that minimize selection pressure on tick populations. Standardized dose-mortality assays and the discriminating dose tests based on them are useful diagnostic tools, but biochemical or molecular genetic assays that provide rapid results and which can be used to estimate the frequency of resistance genes in a tick population, would be a major improvement. In spite of perceptions created primarily by theories about resistance management, there is no basis for expectations of avoiding the development of resistance or of causing resistant populations to revert to a more susceptible state once resistance has evolved. Some theoretical approaches to the management of resistant tick populations may be sound, but data to validate the assumptions on which they are based are rarely available. Also, trials to demonstrate the utility of the strategies have not been done or experimental results have not been transferred to producers. Validated, practical tick control strategies that minimize the number of acaricide applications and integrate other control approaches such as anti-tick vaccines and tick-resistant cattle breeds are needed by producers.

Introduction

The array of acaricides developed and marketed by animal health companies during the last half century resulted in part from competition to invent products efficacious against ticks, but with characteristics such as low mammalian toxicity, little or no detectable residues in milk or meat, minimal persistence in the environment, minimal adverse impact on non-target species, and, from what Nolan (1) referred to as the, "---illusory economic benefits of residual persistence." However, more than any of the characteristics listed above, the effort by the animal health industry to develop new chemical groups of acaricides has been stimulated by the need for new chemistries to overcome problems created by the evolution of resistance to acaricides by ticks. Wherever cattle have been treated repeatedly and frequently with acaricides, resistance of ticks to these chemicals has occurred.

In this review of tick resistance to acaricides various facets of the subject will be covered briefly to provide an overview of the status of the problem and the technology available for sustainable interventions.

The Occurrence of Acaricide Resistance in Ticks

The presence of arsenic resistant strains of *Boophilus* ticks was documented in South Africa as early as 1941, then subsequently in Africa, Australia, South America, and Jamaica. Resistance to chlorinated hydrocarbons, organophosphates (OP=s), and carbamates had been reported in Australia, much of South America, and South Africa for *B. microplus*, and in South Africa for *B. decoloratus* by the early 1960's. Resistance in *Rhipicephalus appendiculatus* and *R. evertsi* populations had been recognized in East and South Africa by 1965 (2). Predictably, the magnitude of the problem with resistance has continued to increase in terms of geographic distribution, numbers of acaricides involved, and number of tick species. In Solomon=s (3) review of acaricide resistance in ticks, most reports of resistance in multi-host ticks involved organochlorine acaricides other than DDT. Accounts of resistance to OP=s were limited to *Amblyomma hebraeum, R. evertsi*, and *R. appendiculatus* in South Africa, and several *Hyalomma* species in the former Soviet Union. Significant additions by Solomon to the list of occurrences of acaricide resistance in ticks included resistance of *B. microplus* in Australia to amidines and permethrin. Even though resistance of *B. microplus* to amidines was first detected in Australia in 1981, resistant populations of this AUlam@ strain are known only from a few widely spread regions in the tick-infested part of the country (4). Another amidine resistant strain (AUltimo@) that is also resistant to all the pyrethroid (P) acaricides was rare, but during the last 4 to 5 years the rate of diagnosis of new cases of amitraz resistance in Australia has accelerated and most of the cases involve the Ultimo phenotype (5). There are now reports of amitraz-resistant strains of *Boophilus* spp. in South Africa (6) and *B. microplus* in Brazil (7). It is noteworthy that the emergence and spread of resistance to amitraz has been slow, but the recent increase in the rate of spread of the polyresistant Ultimo phentogype in Australia is cause for concern.

In contrast to the amidines, the evolution of resistance to P acaricides has been rapid and strains of *B. decoloratus* in South Africa and *B. microplus* throughout much of its geographic range have evolved broadspectrum resistance to these chemicals. Kunz and Kemp (4) observed that twelve years after P acaricides had been introduced in Australia, as a solution to anticipated problems with amitraz resistance, amitraz is being used to control P-resistant ticks.

Diagnosis of Resistance in Ticks

Reliable diagnostic tools are required for determining if acaricide resistance is responsible for tick control failures, selecting an alternative product when resistance is diagnosed, and research to develop tick control strategies that minimize selection pressure on tick populations. The use of diagnostic tools to assess problems with acaricide resistant ticks is often limited to confirming that resistance is responsible for a control failure and providing a rational basis for selecting an alternative acaricide. It is not just important to diagnose resistance on individual premises, but knowledge of the distribution of resistant tick populations is also necessary for making sound choices of acaricides. If the Australians had assumed that Ulam and Ultimo resistant strains of *B. microplus* were widespread instead of focal, they might have failed to take advantage of the efficacy of amitraz for controlling widespread OP- and P-resistant populations (4). After resistance of *B. microplus* to OP=s was documented in Mexico (8) and Federal regulatory authorities allowed P=s to be marketed, the use of P=s became widespread (9), and many producers may have switched to P products even though resistance to OP=s was not a problem for them.

A variety of bioassay methods have been developed, including the larval packet test (LPT), larval immersion test (LIT), filter paper residue test, pipette test, and tea bag test for estimating the susceptibility of larval ticks to acaricides (10). The female dipping test (Drummond method) (11), and adult immersion test (AIT) (D. Kemp, personal communication) are bioassays for determining the susceptibility of engorged females. The LPT and the LIT are the most frequently used bioassay methods and the relative merits of the two techniques have often been the subject of discussions. The LPT was recommended by the Food and Agricultural Organization of the United Nations (FAO) (12) as a standard method for assessing the susceptibility of ticks to acaricides, but it has not yet become the standard method worldwide.

Probit analyses of the dose-mortality responses of susceptible and resistant tick strains provide a basis for characterization of patterns of resistance. When synergists are combined with acaricides it is possible to make inferences about mechanisms of resistance (13). Dose-response data also provide the basis for selecting diagnostic doses that are used in investigations of populations of ticks to determine if they are susceptible or resistant to an acaricide. In such situations, once dose-mortality data are collected for a given chemical, the results of a probit analysis may be used for selecting a diagnostic dose (14), (9). A diagnostic dose must be selected with care to minimize the risk of overlooking resistance because the dose kills a significant proportion of resistant ticks or is so low that many susceptible individuals survive. Unless resistance in the target population has been fully investigated, the LD_{99} is a good dose to select instead of using a diagnostic dose 2- to 3-fold greater than the LD_{99}. Once sufficient data have been collected, a more precise diagnostic dose can be established (15). Not only is it important to have a diagnostic dose that accurately discriminates between resistant and susceptible genotypes, but the size of the test sample is also important for obtaining a useful estimate of the frequency of resistant individuals in a population. The more precise the diagnostic dose and the larger the sample size, the greater the probability of estimating the frequency of resistance genotypes in the population (16).

There are limitations to the existing bioassays. They work well for the, organophosphates-carbamates, and pyrethroids, but reliable bioassays to determine the susceptibility of ticks to acaricides such as amitraz, the macrocyclic lactones, fluazuron, and fipronil have not been published. Another problem with the bioassays is the time required to obtain results. With a one-host tick such as *Boophilus microplus*, a minimum of about 35 days is required after engorged females are collected and larvae of the appropriate age (7-14 days) are available for testing. If a technique such as the AIT (D. Kemp, personal communication) is used, results can be available in 10 days if a sample of engorged females is obtained from untreated cattle.

Existing bioassay methods such as the LPT and the AIT are useful tools for characterizing and diagnosing acaricide resistance in a tick population, but biochemical assays that could enable rapid identification of the resistance phenotype or genotype of individual ticks would be useful. The kinds of biochemical assays that are currently available include: 1) the use of model substrates to detect resistance-related enzyme activity in unprocessed insect extracts, 2) the use of antisera specific for an enzyme to identify the presence of enzymes that confer resistance, and 3) the use of molecular genetic assays to detect specific DNA sequences related to a resistant genotype (17).

There has been progress in research on the molecular genetics of ticks that supports efforts to develop molecular genetic assays such as polymerase chain reaction (PCR) assays for monitoring a tick population to determine the frequencies of resistant individuals. Baxter and Barker (18) isolated the cDNA of acetylcholinesterase (AChE), the target of OP and carbamate acaricides, from *B. microplus* in Australia and compared sequences of OP-susceptible and OP-resistant strains of the tick. They did not detect any point mutations in the putative AChE gene from an OP-resistant strain and concluded that resistance to OPs in Australian *B. microplus* is not conferred by a mutation in alleles of the gene. Hernandez et al. (19) also did not find mutations in the cDNA of AChE cloned and sequenced from OP-resistant *B. microplus* from Mexico. The putative AChE cloned by Hernandez et al. (19) had only 33% identity with the sequence reported by Baxter and Barker (18). This is not surprising in view of the heterogeneity of AChE demonstrated by Nolan et al. (20) whose data suggested at least two forms of the enzyme in *B. microplus.*

Miller et al. (13) used dose-response assays with acaricides and synergists to characterize two patterns of P resistance from *B. microplus* strains collected from Mexico. One pattern was characteristic of a sodium channel mutation and the other involved esterase and cytochrome P450 enzyme systems. He et al. (21) were able to sequence a portion of the cDNA of the sodium channel gene of *B. microplus*, and in two strains previously characterized as having the sodium channel of mechanism resistance to Ps (13), they identified a point mutation of the sodium channel gene. Two different PCR assays for detecting the mutant sodium channel gene in *B. microplus* have been developed (F. Guerrero, personal commun. and A. Chen, personal commun.) and one of them (Guerrero, personal commun) was used to determine the frequencies of the mutant sodium channel allele in samples of susceptible and P-resistant ticks from Mexico. Hernandez (22) obtained two cDNA sequences with a high degree of homology to carboxylesterase B from strains of *B. microplus* from Mexico. One of the sequenced genes had a mutation that was present only in a strain (Coatzacoalcos) shown previously (13) to have a metabolic resistance mechanism involving esterases. Cooperative research between the United States Department of Agriculture, Agriculture Research Service laboratory in Kerrville, Texas, and Texas A&M University in College Station, Texas, is investigating the relationship between the mutant esterase and metabolic resistance of *B. microplus* to P acaricides. The mutant esterase gene occurs in all the larvae of the Coatzacoalcos strain tested and it appears that the PCR assay developed to detect the mutant gene will be a useful diagnostic tool for *B. microplus* with metabolic resistance to Ps.

It is likely that molecular genetic and/or biochemical assays will soon be available for diagnosis of P and OP resistance in *B. microplus* populations and it will be possible to use these assays in Mexico in investigations of the epidemiology of resistance. The assays can also be used in other countries to determine the geographic distribution of the resistance mechanisms that have evolved in *B. microplus* populations in Mexico. Similar diagnostic methods are needed for amitraz, macrocyclic lactones, fluazuron, fipronil, and any new products with novel modes of action. If these novel diagnostic methods are to be useful, it would be advantageous to have them available as quickly as possible before newly evolved resistance mechanisms emerge. Research on the diagnosis of resistance to amitraz should be a priority. Approaches such as the use of ethyl methane sulphonate (EMS) mutagenesis (23 (24) could aid in the discovery of resistance mechanisms that may evolve in response to selection pressure by new acaricides.

Mitigation of Resistance of Ticks to Acaricides

Goals for resistance management programs have been defined as implementing measures to: a) overcome the loss of control related to a resistant tick population; b) avoid resistance development in tick populations; and c) slow the rate of resistance development (25) (1). It is unlikely that it will be possible to prevent the evolution of resistance as a consequence of acaricide applications, but there are options for slowing the rate of resistance development and there are a few options that may be exercised when resistance renders a pesticide ineffective.

Once acaricide resistance has evolved in a tick population the choices for addressing the problem are continuing to use the acaricides affected, switching to a chemical to which the individuals in the population are susceptible, or eradication (1). Eradication of a focal occurrence of resistance is most likely to succeed when: a) the distribution of the resistant strain is limited to a small number of premises; b) the target is a species like *B. microplus* with a high degree of host-specificity; c) an effective alternative acaricide is available; and d) there is a strong commitment to eradication by producers and government. A quarantine to prevent animals infested with resistant ticks from dispersing and spreading resistant ticks would also be critical to the success of an eradication program (26). Other options for extending the useful life of a group of chemicals are to increase the concentration or if resistance is due to a metabolic mechanism, adding a synergist to the acaricide formulation is a possibility (1). It may not be necessary or even advisable for producers to abandon a product once resistance to it is documented because resistance is usually not ubiquitous and a product useless in one area may still be effective elsewhere. Once resistance to P acaricides began to emerge, it rapidly became widespread, but in the two decades since amitraz resistance was reported in Australia the distribution of resistant populations has remained relatively restricted (4).

Roush (27) emphasized that of the fundamentals to consider in the design of any resistance management program, the initial and most important step is to reduce the number of pesticide treatments. It is also important to recognize that a strategy, which otherwise provides optimal tick control may not be optimal in terms of resistance management. Sutherst and Comins (28) illustrated the advantage of commencing a control program in the spring to impact the tick population at a time when the greatest proportion of it will be in the parasitic phase. The conclusion by Georghiou and Taylor (29) that "All else being equal, it is desirable to treat a population before its numbers get too large,@ also seems to support early spring treatments as a beneficial resistance management practice. Unfortunately, this approach appears to be in conflict with the concept of maintaining a reservoir (refugia) of untreated individuals (30). Selection for resistance genotypes may be delayed if refugia of untreated cattle provide an opportunity for competition between resistant and susceptible ticks. How can refugia be maintained in the context of a tick control program? Instead of beginning to treat cattle as soon as ticks appear on animals, an economic threshold policy could be used to correlate the initiation of the treatment cycle with the appearance on cattle of a tick density that equals the economic damage threshold (28). A second way of providing refugia for susceptible ticks would be to exclude from treatment those animals on which the tick burden does not exceed the treatment threshold. Implementation of this tactic would be facilitated in herds of tick-resistant Zebu or Zebu-cross cattle instead of European breeds (31). Tactics that minimize the number of treatments and which reduce the proportion of a herd that is treated reduce the selection pressure for resistance.

For the control of *Boophilus* ticks, Sutherst and Comins (28) recommended treating at three-week intervals. A longer interval between treatments may be possible with chemicals that provide a residual protective period, but following the initial high dosage the decay of the residual acaricide may select for resistant heterozygotes, and the short-term benefits will be offset by loss of product effectiveness because of the development of resistance (31). The choice of acaricide dose concentration is another operational decision that can affect the selection of individuals with resistance genotypes and models have been used to test hypotheses about the effects different concentrations (30), (28). Theoretically, when selection pressure in the form of acaricide treatments is first applied to a tick population in which there are mutants with characteristics that favor their survival under these conditions, individuals that are homozygous for the trait that confers resistance are assumed to be rare. A high rate of selection for resistance results from treating cattle with an acaricide dose that kills most homozygous susceptible ticks, but permits survival of resistant heterozygotes. Selection is much more rapid if the resistance alleles are partially dominant than if the alleles are incompletely recessive. According to models, the optimal strategy is to neutralize the competitive advantage of the heterozygous-resistant individuals by applying a dose with a concentration that is sufficient to control the heterozygotes. While the high dose strategy is sound theoretically, it suffers from significant limitations when applied to the control of pesticide-resistant arthropods (27). Roush listed the deficiencies of high dose tactics as: Aeconomic and environmental limitations on doses needed, the difficulty of maintaining doses high enough to kill heterozygotes, the deleterious effect of pesticide residues on the inward migration of susceptible insects, and the difficulty of maintaining an untreated source for immigrants". Perhaps one of the greatest flaws of the high-dose strategy is that the dose needed to kill resistant heterozygotes is unknown. Even if the proper dose was known, uneven coverage of treated animals and the decay of residual acaricide would result in a dose too low to cause the 95% mortality rate needed if the tactic is to be successful. Also, the strategy must be applied when the resistance allele frequency is less than 10^{-3} and it is improbable that this knowledge would be available. In relation to a low dose approach, Roush (27) referred to what he called the Apersistent myth that resistance can be managed by low doses@ while rejecting the assertion that low doses in themselves can be an effective part of a resistance management strategy.

When two or more acaricides that have unique modes of action and which do not have the potential for cross-resistance are available, consideration may be given to treatment strategies involving their use in combination (mixtures), or by alternating their use over time (rotation) (31), (32), (27). The assumption that is the basis for a rotation scheme is that the frequency of individuals resistant to one acaricide will decline during the time that the alternate chemical is used. Whether a decline occurs in the frequency of ticks with resistant genotypes depends on the occurrence of negative cross-resistance and a low degree of fitness of resistant individuals (32). Rotation may offer a theoretical advantage in models or laboratory experiments, but this tactic needs to be evaluated in the field. A rotation strategy may succeed in some situations, but it is not possible to generalize in the absence of supporting data.

Assumptions for using mixtures are that: resistance to each pesticide is monogenic; there is no cross-resistance; each pesticide is equally persistent; resistant individuals are rare; and that some of the target population remains untreated (32). The use of mixtures in an effort to delay resistance is perhaps the most controversial of all the proposed resistance management strategies. Positive results with laboratory trials may not reflect performance in the field and the lack of a positive control in the field prevents documentation of what would have occurred with both compounds in the absence of the mixture. The criteria of matching pesticides with equal persistence and implementing a mixture strategy while resistance to both chemicals is low are both challenging conditions (33).

The Mitigation of Resistance and Integrated Pest Management

Past experience provides no basis for optimism that we can avoid resistance, but we must implement rational control programs that delay its adverse impact. What are the characteristics of a rational control program? Hoy (34) reasons that management of pesticide resistance in pest arthropods and integrated pest management should not be perceived as distinct topics, but should be considered to have equivalent goals and methods. She points out that, "Effective management of resistance and effective IPM programs require an holistic and multitactic strategy@. Treating cattle with an acaricide is likely to remain the central feature of tick control programs, but selection pressure for characteristics that confer resistance can be moderated by incorporating elements into control strategies that enable producers to reduce the number of times they need to apply an acaricide to their tick-infested cattle.

Options available for incorporation with acaricides into a multitactic tick control program are: tick-resistant breed of cattle, anti-tick vaccines, host management, and pasture vacation (35) (36). Few cattle raisers have the quantity of grazing land or management systems needed to implement host management and pasture spelling approaches. Stocking with >tick-resistant= cattle breeds and vaccination of cattle with an anti-tick vaccine are the two options most likely to be adopted by producers. Australians pioneered investigations that documented differences within and between breeds of cattle to acquire protective resistance to parasitism by ticks. They capitalized on these observations by developing schemes for using tick-resistant cattle as a component of *B. microplus* control programs (37). The value of cattle breed-associated acquired resistance to tick infestations has also been demonstrated in East and Central Africa against multi-host tick species (38), (39). The first commercialization of an anti-tick vaccine (TICKGUARD^{TM}) by Australian scientists and the subsequent marketing of a similar vaccine by Cubans (Gravac^{TM}) provided new alternatives for *B. microplus* control programs. Both vaccines can be used as major components in tick management programs to reduce the number of acaricide treatments needed (36). Either one or both of these imunological-based methods for controlling ticks could be used in conjunction with acaricides to implement tick control tactics that would require limited use of acaricides to keep tick numbers on cattle below economic thresholds and would extend the useful lives of acaricides.

Discussion

As resistance to acaricides becomes a progressively more widespread and complex hindrance to cost-effective cattle production, producers need to employ tactics that help them achieve their economic goals and reduce the rate at which ticks are evolving resistance. Before they can improve the ways in which they approach the problem of controlling ticks, they must have accurate information and clear guidelines from scientists and extension specialists. There is a large body of excellent scientific literature on all aspects of tick control, including the detection and management of resistance, but much of it is theoretical and cannot be applied to specific problems. Knowledge of factors, such as mechanisms of resistance, inheritance of resistance, dose-response relationships, and comparative fitness of resistant and susceptible ticks in relation to specific acaricides, is unavailable. There are also few practical trials that demonstrate the economic value of different tactics.

What tools and alternatives are available? Bioassays to determine dose-response relationships and to estimate discriminating doses are still the key to resistance diagnosis. We need to adopt standard methods and procedures for obtaining and reporting resistance data. The FAO Larval Packet Test is useful for many chemicals, but standard methods for diagnosing resistance to amitraz and new groups acaricides are needed. Biochemical and molecular genetic assays may change the way resistance is diagnosed in the future, but the usefulness of these kinds of tools remains to be proven. The methods of molecular genetics are helping to resolve important questions about resistance mechanisms. In spite of theories about how to reduce the rate at which resistance evolves and how to manage populations in which resistance has invalidated the use of a specific chemical or group or chemicals, there is a single clear guideline to recommend. Use acaricides as little as possible. An economic threshold approach to initiating acaricide treatments, excluding animals with light tick burdens from treatments, stocking with tick-resistant cattle, and vaccinating cattle with anti-tick vaccines are the key options available. Guidelines producers can use to design and implement profitable tick control programs can only be developed after scientists do the applied research needed to inform us about the best ways to use available technology under different conditions. Research to develop new and improved diagnostic tools, to improve resistance management approaches, and to develop new and improved control technologies is needed.

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Keywords

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