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Allele Specific PCR as a diagnostic tool for detection of pyrethroid resistance in cattle ticks

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Abstract
Ticks are major ectoparasites of livestock and cause significant economic losses through blood feeding and transmission of pathogens (Jongejan & Uilenberg, 2004). Synthetic pyrethroids are widely used for tick control, but their extensive use has resulted in resistance in *Rhipicephalus microplus* populations (George et al., 2004). Mutations in the voltage-gated sodium channel gene lead to knockdown resistance against pyrethroids (Soderlund & Knipple, 2003). Allele-specific polymerase chain reaction (AS-PCR) is a rapid molecular technique used to detect resistant and susceptible alleles in tick populations (Guerrero et al., 2001). This method enables early detection of resistance and supports effective monitoring and management of acaricide resistance in field populations (Abbas et al., 2014).

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INTRODUCTION

Among the most economically significant ectoparasites affecting livestock globally are ticks. Through blood feeding, they inflict direct harm and indirectly spread a number of infections that cause tick-borne illnesses such theileriosis, anaplasmosis, and babesiosis (Jongejan & Uilenberg, 2004). *Rhipicephalus microplus*, a cow tick that causes large productivity losses in the livestock industry, is especially important in tropical and subtropical areas (Grisi et al., 2014). The major technique for controlling ticks is still chemical acaricides. Among them, synthetic pyrethroids like flumethrin, cypermethrin, and deltamethrin are frequently employed because of their low environmental persistence, minimal mammalian toxicity, and great efficacy (George et al., 2004). However, many tick populations have developed broad acaricide resistance as a result of the frequent and heavy use of these drugs (Abbas et al., 2014). Pyrethroids exert their toxic effects by targeting the voltage-gated sodium channels of nerve cells, disrupting normal nerve impulse transmission and causing paralysis in ticks (Soderlund & Knipple, 2003). Resistance to pyrethroids often occurs due to mutations in the sodium channel gene, which reduce the sensitivity of the channel to these compounds. This mechanism is commonly referred to as knockdown resistance (kdr) (Dong, 2007).

PROCEDURE

Ticks that may be resistant to pyrethroid acaricides are obtained from cattle afflicted in various regions. For molecular investigation, adult female ticks that are engorged are usually utilized. The ticks are kept at −20°C until DNA extraction after being properly cleaned with ethanol and distilled water to get rid of impurities (Morgan et al., 2009).Standard phenol-chloroform extraction techniques or commercially available DNA extraction kits are used to recover genomic DNA from individual ticks. For additional molecular analysis, the isolated DNA is measured and kept at -20°C.To find mutations linked to pyrethroid resistance in the voltage-gated sodium channel gene, allele-specific PCR is used. Based on known point mutations, certain primers are made to distinguish between susceptible and resistant alleles. During PCR amplification, these primers can distinguish between the wild-type and mutant alleles because they bind to them selectively (Guerrero et al., 2001). Template DNA, forward and reverse primers specific to susceptible and resistant alleles, deoxynucleotide triphosphates (dNTPs), buffer solution, magnesium chloride, and Taq DNA polymerase are often included in the PCR reaction mixture. A thermocycler is used to perform amplification under ideal cycling circumstances, which include initial denaturation, several cycles of denaturation, annealing, and extension, and a final extension phase. After being stained with ethidium bromide or other nucleic acid dyes, the amplified PCR products are separated using agarose gel electrophoresis and seen under ultraviolet light. The tick's sensitive, resistant, or heterozygous alleles are shown by the presence or lack of particular amplification bands.

DISCUSSION

A useful molecular technique for identifying pyrethroid resistance in ticks is allele-specific PCR. AS-PCR enables quick identification of resistance-associated mutations at the DNA level, in contrast to conventional bioassays that need for a lot of live ticks and lengthy testing times. High sensitivity, specificity, and the capacity to identify resistance even in tick populations with low frequencies of resistant alleles are just a few benefits of this approach (Guerrero et al., 2001). Additionally, it can distinguish between genotypes that are susceptible, heterozygous, and homozygous resistant, which is crucial for comprehending the genetic makeup of resistant populations. Early monitoring and surveillance operations are also made easier by molecular identification of resistance. Early detection of resistant populations allows for the implementation of suitable control strategies including integrated tick management, acaricide rotation, and less reliance on chemical control (Abbas et al., 2014).

CONCLUSION

By identifying mutations in the voltage-gated sodium channel gene, allele-specific PCR is a useful molecular diagnostic method for identifying pyrethroid resistance in ticks. The method is crucial for tracking acaricide resistance in tick populations because it offers quick, accurate, and sensitive resistance allele detection. Incorporating molecular diagnostic methods such as AS-PCR into tick surveillance programs can greatly enhance the effectiveness of integrated tick management strategies and help reduce economic losses associated with tick infestations and tick-borne diseases.

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