PASA assay for diagnosing pyrethroide resistance in the cattle tick populations in Rondônia - Brito L.G.[1]*, Barbieri F.S.[1], Oliveira M.C.S.[2], Guerrero F.D.[3]

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Knockdown (kdr) resistance in field populations of cattle tick can severely limit pyrethroid usefulness in tick control programs. Early detection and characterization of kdr resistance are critical to the development of resistance management strategies. Cattle tick samples collected in Porto Velho and Presidente Medici, Rondonia were analyzed at Knipling-Bushland U.S. Livestock Insects Research Laboratory to verify the genotypes of these cattle tick populations. The populations were assessed using commercial cypermethrin by Adult Immersion Test (AIT). Engorgement females ticks were exposed for 30 minutes in cypermethrin solution prepared according to the manufacturer recommendations. The control group was formed with engorged females, which were immersed in distilled water. Three groups of ten ticks were used in each treatment. Ten females by Presidente Medici population and three females by Porto Velho population immersed in commercial cypermethrin solution survived. The survived females were placed in B.O.D to obtain the postures. After the eggs hatch, the larvae were collected and immediately frozen at ultra-low temperature. Genomic DNA was isolated from individual larvae cattle tick and 30 larvae of each population tested by PASA (PCR amplification of specific alleles) assay for the presence of a specific nucleotide substitution in the sodium channel gene that has been associated with kdr resistance in cattle tick. The kdr allele was detected only in Presidente Medici population, which was considered a pyrethroid heterozygous (SR) population. This result was expected for this cattle tick population that showed a resistance factor of 18.35 for the pesticide evaluated.

Key-words: molecular detection, pesticide resistance, *Rhipicephalus microplus*

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PASA ASSAY FOR DIAGNOSING PYRETHROID RESISTANCE IN THE CATTLE TICK POPULATIONS IN RONDÔNIA

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Introduction

The cattle tick, *Rhipicephalus (Boophilus) microplus*, causes major economic losses to cattle producers in many parts of the world, both directly from physical effects upon infested animals and indirectly through the diseases caused by protozoan parasites transmitted during the tick's bite. In Brazil alone, the losses have been estimated at more than US$ 2 billion annually (Grisi et al., 2002). Currently, the level of resistance in a tick population is determined by bioassay techniques. The most commonly used protocols for assaying females are the Adult Immersion Test (AIT) and testing with filter paper packets. The bioassay procedures are valuable for their portability and low cost. However, these tests do not reveal any information about the sample populations' genetics, cannot detect resistance in its early stages of development, and often take days or weeks before results can be determined. Significant delays occur since oviposition and hatching must occur before the bioassays can be started. An assay that could determine the resistance status of single ticks within a day would be helpful. With this goal in mind, Guerrero et al. (2001) examined the feasibility of developing a polymerase chain reaction (PCR)-based assay capable of detecting pyrethroid target site resistance in single ticks. Knockdown (kdr) resistance is caused by a reduction in the sensitivity of the arthropod nervous system to pyrethroids. The sodium channel is the target of pyrethroids. Single nucleotide substitutions in the S6 transmembrane segment of domain II and the S4-S5 loop of domain II of the sodium channel gene have been directly linked to pyrethroid insensitivity (Lee et al., 1999). Kdr resistance in field populations of cattle ticks can severely limit pyrethroid usefulness in tick control programs. Early detection and characterization of kdr resistance are critical to the development of resistance management strategies.

Materials and Methods

Cattle tick samples collected in ranches in Porto Velho and Presidente Medici, Rondônia, were analyzed at the Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA/ARS, Kerrville, TX, to verify the genotypes of these cattle tick populations. First, the populations were assessed using commercial cypermethrin solution by AIT conducted at the Embrapa Rondonia Animal Health Laboratory. Engorged female ticks were exposed for 30 minutes to a commercial cypermethrin solution prepared according to the manufacturer's recommendations. The control group was formed of engorged female cattle ticks belonging to each of the populations tested, which were immersed in distilled water. Three groups of ten ticks were used in each treatment. Ten females from the Presidente Medici population and three from the Porto Velho population immersed in the commercial cypermethrin solution survived. The surviving females were placed in B.O.D to obtain the postures. After the eggs hatched, the larvae were collected and immediately frozen at ultra-low temperature. Genomic DNA was isolated from individual tick larvae and 30 larvae of each population were tested by the PASA assay (PCR amplification of specific alleles) for the presence of a specific nucleotide substitution in the sodium channel gene sequence that has been associated with kdr resistance in cattle ticks. The primers FG 221 and FG 227 (reaction 1) or FG 222 and FG 227 (reaction 2) produced diagnostic products for genotyping the kdr allele. Reaction products were visualized by 4% agarose gel electrophoresis followed by UV illumination after staining with Syber Green.

Results and Discussion

In the PCR assays of these two strains, all individuals were homozygous susceptible except for one heterozygote, which was detected in the Presidente Medici strain. This result was expected for this cattle tick population, as there is a report that this population showed a resistance factor of 18.35 for the pesticide evaluated. This is the first report of kdr alleles in cattle tick populations in Rondônia. The climatic conditions in the humid tropics are very favorable for the development of cattle ticks throughout the year (Brito et al., 2009). For this raison, most dairy farmers in the state treat their herds with pesticide solutions. The presence of kdr alleles in the President Medici population showed that the resistance to pyrethroid pesticides may also be occurring in other cattle tick populations in Rondônia. Further studies to verify the prevalence of kdr alleles in populations of *R. microplus* in Rondônia are necessary to establish management strategies for avoiding tick fixation of resistant alleles in the cattle tick populations in Rondônia.

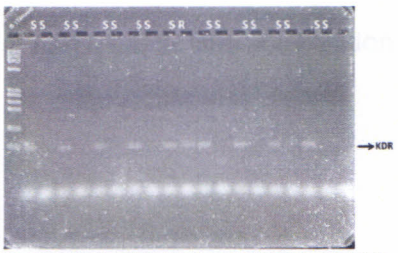

Figure 1: Agarose gel stained with 4% GelStar with the PCR products of DNA samples of the larvae of cattle tick population genotyped for KDR mutation, which confers resistance to pyrethroid pesticides. Where: gel 2.1% Nae III = standard base pairs; SS = samples of larvae with genotype for susceptibility; SR = sample of heterozygous larvae from the Presidente Medici strain; KDR = band of 68 bp, which enables diagnosis of the kdr mutation in *Rhipicephalus (B.) microplus* populations.

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