



AR145

PASA ASSAY FOR DIAGNOSING PYRETHROIDE RESISTANCE IN THE CATTLE TICK POPULATIONS IN RONDONIA

BRITO, L.G. 1; BARBIERI, F.S. 1; OLIVEIRA, M.C.S. 2; GUERRERO, F.D. 3

1 Embrapa Rondônia, Porto Velho, RO, Brazil. 2. Embrapa Cattle-Southeast, São Carlos, SP, Brazil. 3. Knipling-Bushland U.S. Livestock Insects Research Laboratory, USDA/ARS, Kerrville, TX, US.

Knockdown (kdr) resistance is caused by a reduction in the sensitivity of the arthropod nervous system to pyrethroids. 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, USDA/ARS, Kerrville, TX, to verify the genotypes of these cattle tick populations. First, the populations were assessed using commercial cypermethrin solution by Adult Immersion Test (AIT) conducted at Embrapa Rondonia Animal Health Laboratory. Engorgement females ticks were exposed for 30 minutes in commercial cypermethrin solution prepared according to the manufacturer recommendations. The control group was formed with engorged females 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 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 sequence that has been associated with kdr resistance in cattle tick. 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 after 4% agarose gel electrophoresis followed by UV illumination after staining with Syber Green. 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 as there is report of resistance to pyrethroid pesticides, since the population showed a resistance factor of 18.35 for the pesticide evaluated.

Palavras-chave: Pyrethroid resistance, *Rhipicephalus microplus*, kdr, allele characterization, Rondonia.