



DNA from collected ticks was subjected to PCR amplification targeting genes previously linked to acaricide resistance, namely the octopamine/tyramine receptor and the voltage-gated sodium channel. SNP detection was performed via sequence similarity analyses, and phylogenetic relationships were established using Maximum Likelihood algorithms. The study identified 13 commercial acaricide brands in use, with most farmers (90.6%, 348 out of 384) applying amitraz up to twice weekly. Approximately 60% (230 out of 384) of farmers rated acaricide efficacy as “Very Good,” and 35% (136 out of 384) rated it as “Good,” indicating that 95% of farmers were satisfied with acaricide performance. Larval packet tests revealed that tick populations were resistant to at least one of the five amitraz molecules and exhibited multi-acaricide resistance—contradicting farmers’ positive perceptions. While tick populations remained susceptible to synthetic pyrethroid chemicals in laboratory tests, genetic analysis revealed a concerning mutation. A non-synonymous mutation in the domain II S4-5 linker region of the para-sodium channel gene was found in one field sample. This mutation, previously linked to acaricide resistance in other countries, indicates that ticks in Burundi are developing resistance to synthetic pyrethroids. Frequent acaricide application has led to the emergence of acaricide-resistant *R. microplus* in Burundi. While most farmers perceive their tick control as effective, our findings highlight early signs of resistance. These results underscore the need for integrated tick management strategies – including rotation of acaricide classes, farmer training in proper use, and alternative control measures – to sustain effective tick control and mitigate tick-borne diseases.

Validation of a New Artificial Intelligence-Based Technology for Predicting Acaricidal Efficacy against *Rhipicephalus microplus*

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This study aimed to validate a new artificial intelligence-based technology for predicting the efficacy of acaricidal products against *Rhipicephalus microplus*. Engorged females from 20 different populations were subjected to the adult immersion test, being divided into 15 experimental groups (n = 10): one untreated control group and 14 treated groups with different acaricides, applied at single and specific concentrations as indicated by the manufacturers. After the incubation period, 0.1 g samples of eggs with 14 days of oviposition were collected from each treatment and population for automated efficacy analysis. The eggs were placed in Petri dishes and photographed using a smartphone coupled to a binocular stereoscopic magnifying glass. The images obtained were used to estimate the percentage of larval hatching and calculate the control percentage (C%) through the automatic model. The validation consisted of comparing the C% automatically generated by the software with those obtained manually in the adult immersion tests, through the analysis of three statistical metrics: RMSE (Root Mean Square Error), MAE (Mean Absolute Error), and R² (Coefficient of Determination). The obtained RMSE was 20.76, the MAE was 16.28, and the R² reached a value of 0.74, indicating that the



model was able to explain 74% of the variability of the real data. The results demonstrate that the deep learning model shows satisfactory performance and potential for use as an auxiliary tool in estimating tick control, being considered validated for preliminary use. However, the expansion of the database is necessary to increase the robustness and applicability of the predictive system. This new method accelerates result acquisition by up to 16 days, half the time required by the traditional method.

Evaluation of a Rapid Alternative for Resistance Diagnosis in *Rhipicephalus microplus*

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The cattle tick *Rhipicephalus microplus*, a vector of pathogens in bovines, causes estimated annual losses of R\$ 350 million to the livestock industry in Rio Grande do Sul, Brazil. The increasing resistance to acaricides worsens the situation, making effective control more difficult and costly. Resistance diagnosis through bioassays such as the Adult Immersion Test (AIT) is essential for guiding treatment strategies. However, the standard AIT takes 42 days, due to the need to wait 14 days for oviposition and 28 additional days for larval hatching. This study aimed to evaluate whether results based solely on egg mass production at 14 days differ significantly from the full AIT, potentially allowing resistance diagnosis to be anticipated by up to four weeks. For each acaricide formulation - cypermethrin, deltamethrin, cypermethrin+chlorpyrifos, and amitraz - the efficacy variables based on oviposition (EIP) and fertility (EIF) were compared using Pearson correlation analysis. Additionally, agreement between

the standard and alternative tests was assessed using Cohen's kappa statistic based on efficacy categories (high, moderate, or low). The overall Pearson correlation between EIP and EIF was 0.9273, indicating a strong correlation. High correlations were also observed individually: cypermethrin ($r=0.8832$), deltamethrin ($r=0.9457$), amitraz ($r=0.8988$), and cypermethrin+chlorpyrifos ($r=0.8932$). In terms of kappa agreement, cypermethrin ($k=0.7922$) and deltamethrin ($k=0.7832$) showed moderate agreement between the two methods, while amitraz ($k=0.8233$) and cypermethrin+chlorpyrifos ($k=0.8088$) showed strong agreement. These results demonstrate that diagnostic evaluations based on egg mass production at 14 days can serve as a reliable proxy for the full AIT. This approach significantly benefits livestock producers by reducing the time needed for diagnosis, improving responsiveness, and supporting more effective tick control strategies.

A new larval packet test protocol for field screening of fluralaner resistance in *Rhipicephalus microplus*

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Rhipicephalus microplus is considered the most important bovine parasite worldwide. Tick control relies primarily on chemical aca-