RESUMO SIMPLES - ECTOPARASITOS: SISTEMÁTICA, EPIDEMIOLOGIA E CONTROLE

COMPARISON OF HEMOLYMPH DILUTION METHODOLOGIES FOR TOTAL HEMOCYTE COUNT QUANTIFICATION IN RHIPICEPHALUS MICROPLUS.

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The cattle tick, Rhipicephalus microplus, is the most significant ectoparasite to Brazilian livestock farming in terms of economic and sanitary impact. The indiscriminate use of synthetic acaricides for its control has led to the emergence of resistant populations, demanding the development of new strategies. In this context, studying the dynamics of hemocytes—the arthropods' hemolymph cells—is a crucial alternative. Hemocytes, with vital functions in transport and protection/immunity, provide information about the tick's cellular

response to pathological and chemical agents. The Total Hemocyte Count (THC) is an essential parameter for assessing the impact of external aggressors (pathogens or drugs). Knowledge of this cellular response aims to elucidate the immunology and physiological mechanisms involved in potential resistance processes, supporting the implementation of more effective control measures. This study compared two methodologies for diluting the hemolymph of engorged female R. microplus in sodium citrate anticoagulant, using different proportions, to verify their influence on the reliability of the total hemocyte count. Based on this comparison, the goal was to identify the most suitable dilution for standardizing an optimized collection method, capable of ensuring higher accuracy while using a smaller volume of hemolymph for total hemocyte quantification. The Total Hemocyte Count (THC) was performed on engorged Rhipicephalus microplus females from the cattle-maintained colony at the Laboratory of Experimental Chemotherapy in Veterinary Parasitology, approved by CEUA protocol 1268101223. The ticks, recovered on the day of detachment (dropping), were cleaned in running water, bathed in 0.05% hypochlorite for five minutes, dried, and selected into groups of homogeneous weights. Hemolymph collection was performed by dorsal cuticle puncture (0.3 mm needle), and the material was immediately transferred to microtubes with anticoagulant sodium citrate buffer, maintained on ice. THC was determined using a NEW OPTICS® Improved Mirrored Global Neubauer Chamber under optical microscopy (100x). Two comparative assays were performed: the first, with individual collection (each female as one unit), used a 4:1 dilution (8 µL of citrate to 2 µL of hemolymph); the second, with a pool of five females, used a 1:1 dilution (10 µL of citrate to 10 µL of combined hemolymph), aiming for homogeneous volume and reduced variability. The result of the individual count was 342, 218, 536, 108, 273, and 792 cells, and the result of the group count was 692, 1440, 425, 1228, 1240, and 1240 cells. To observe the possible existence of a significant difference between the group and individual sampling, the data were evaluated for normality using the Shapiro-Wilk test. Since the data were parametric, the comparison between the groups was performed using the T-test, and the analyses were conducted in the Bioest program with a 95% confidence interval. The results showed no statistical difference between the methods, with means of 4,93 x 106 cells/ml for the group assay and 4,73 x 106 cells/ml for the individual assay. However, the standard deviation observed in the individual assay was significantly greater, indicating that, although the means between the methods are similar, there is greater biological variability among the teleogynes evaluated individually, while the pooling method tends to produce more

homogeneous values. From this, we can conclude that both evaluated dilution methods are valid for total hemocyte quantification in engorged Rhipicephalus microplus females. Nevertheless, the individual method has the advantage of requiring a smaller hemolymph volume, reducing the need for a high number of teleogynes and making the process faster, as it involves fewer collections. Thus, the choice of the most appropriate method should consider the experimental conditions, the availability of biological material, and the specific objectives of the research, balancing practicality, representativeness, and desired biological variability.

Palavras-chave: ticks; cellular response; teleogynes; neubauer; method.