

## ABSTRACT

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### Morphological and physicochemical analysis of tick harvested from horses treated with a homeopathic complex: an experimental protocol

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Ticks are the most abundant external parasites in the world. Ticks of the *Amblyoma* genus, which parasitize capybaras, equines, and primates, play an important role in public health as they are vectors of zoonoses, such as spotted fever. The proposed protocol was prepared for a study aimed at understanding the effect on tick morphology of a homeopathic complex provided by “SIGO Homeopatia” Laboratories (Campo Grande, Brazil), a certified laboratory by the Brazilian Ministry of Agriculture. It is prepared in a long-term, slow dispersive solid base of calcium carbonate gel, composed of *Psorinum*, *Sulphur*, *Ledum palustre*, *Artemisia lerchiana* 12cH, and *Apis mell* 7cH, on the morphology of *Amblyoma sculptum* adult female ticks, collected from infested horses, treated or not with the complex immersed into the drinking water. Additionally, the identification of specific physicochemical signals of the product in the drinking water and the ticks' hemolymph will be performed using the solvatochromic dye method, according to a previously established methodology [1]. Two groups of 20 horses each, kept on a property located at Jaraguari, MS, Brazil, will be separated into two paddocks. In one of them, the complex dispensed solid base will be immersed in the drinker and, in the other, the inert solid base will be inserted. Both preparations will be codified immediately after their production, and all analyses will be performed blindly, including statistical data analysis. After 15 days of treatment, samples of 10 adult female ticks will be collected per donor animal, totaling 100 individuals per group. The external morphology of each specimen will be analyzed by employing digital images of the dorsal, ventral, and rostral faces. The hemolymph will then be collected by amputating one of the first pair of legs limbs [2]. Two samples will be collected per tick: one drop will be used to make a smear on a histological slide for later analysis of the cellular composition, and the other will be pooled according to each donor and deposited in a microtube containing 30% filtered ethanol for later analysis using the solvatochromic dye method. Another pooled sample will be deposited in a second empty microtube and frozen for possible later epigenetic analysis. The slides will be fixed in PA methanol at 4°C for 15 minutes, stored in a freezer until staining using the Giemsa method, mounted with a coverslip and histological resin, and read under an optical microscope. After the hemolymph is extracted, the same specimens will be perfused with 10% buffered and filtered formalin, sectioned according to the Boin method [3], and processed according to the histologic resin inclusion method and hematoxylin-eosin staining for subsequent histopathological evaluation. This is an original protocol, never tested before, whose project expectation is to demonstrate (or not) possible direct morphological changes in ticks

obtained from animals treated with the complex, thereby contributing to the elucidation of its mechanism of action. Before any intervention, this protocol will be submitted to the animal ethical committee for approval.

**Keywords:** spotted fever, environmental homeopathy, Ixodidae, ectoparasites

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### Acknowledgments and conflict of interest

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### References

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