



Original Article

In vivo study of a homeopathic medicine against *Rhipicephalus (Boophilus) microplus* in dairy cow



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ABSTRACT

The tick *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae) causes large economic losses to cattle breeders and its control is hampered by problems of resistance to the main commercial synthetic acaricides and the risk of residues in animal products. Alternative controls are important for the sustainability of cattle breeding in tropical regions, principally for organic milk production. This study evaluated the efficacy of a homeopathic preparation to control natural tick infestations. The evaluations were conducted with 24 taurine dairy cows during 12 months. The homeopathic medicine (30 CH + Sulfur 30 CH) was administered in a proportion of 1:100 (v/w) in a formulation with sugar, added to 30 kg of mineral salt. This preparation was supplied along with concentrated feed (2 kg/animal/day). The control group received the same feed mixture without the medicine. The groups were kept in separate paddocks. Data were collected on body weight, packed cell volume and number of engorged female ticks on each animal. Temperature, relative humidity and rainfall were recorded. Engorged females were collected from each group for *in vitro* comparison of reproductive parameters, and semi-engorged ticks for morpho-histological analysis. The mean results of body weight, packed cell volume and tick number were not statistically different between the control and treated groups: 616.5 kg and 618.6 kg; 27.9% and 27.3%; and 12.19 and 13.58 ticks, respectively. In the *in vitro* analyses, the average weights of the ticks (0.18 and 0.17 g) and eggs (0.07 and 0.06 g), larval hatching rate (70.1 and 76.6%) and reproductive efficiency index (54.3 and 60.6%) of the control and treated groups, respectively, also were not statistically different. Furthermore, no alteration in the ovaries was detected by microscopic analysis. Therefore, the homeopathic medicine was ineffective to control *R. (B.) microplus*, although the inclusion of agro-ecological practices might enhance its effect of homeopathy in controlling tick infestations.

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Introduction

Populations of *Rhipicephalus (Boophilus) microplus* (Canestrini, 1887) (Acari: Ixodidae) are established in all inter-tropical regions, and the species is considered one of the main obstacles to the profitability of livestock breeding (Higa et al., 2016). In Brazil alone this tick is responsible for estimated yearly losses of US\$ 3.24 billion

(Grisi et al., 2014). Besides causing direct damages to production of meat and milk due to blood spoliation, it also reduces the value of leather because of hide damage and transmits the hemoparasites that cause bovine babesiosis and anaplasmosis, diseases that cause high mortality (Guerrero et al., 2014).

The main way to control this tick is to apply conventional synthetic acaricides (Kunz and Kemp, 1994; Santos et al., 2002), but this leads to selection for resistance among ticks to the toxic effects of these drugs, through different mechanisms (Baron et al., 2015). This problem is becoming more severe, prompting a need to find alternatives to help control ticks (Pazinato et al., 2014), especially due to the alarming development of multiple resistance and the

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shortage of molecules with new modes of action available in the market (Raynal et al., 2015; Klafke et al., 2017).

Organic stock breeders place priority on phytotherapeutic and homeopathic methods to control parasites (Osterroht et al., 2002). Besides this, the indiscriminate use of conventional veterinary drugs can cause the presence of residues in animal products and the environment. To minimize this risk, alternatives must be found to substitute or at least reduce the application of these drugs for parasite control, to assure the sustainability of production systems and food safety of the population (Braghieri et al., 2007; Bedi et al., 2015).

Homeopathy acts in different ways, according to the main needs of each individual of the herd or the population treated. The levels of action that have been best elucidated are: stimulation of the organism's defense, improved productivity and quality of final products, drainage (elimination of endogenous and exogenous toxins), and interference in quorum sensing (Real, 2008). In this respect, homeopathy can be an alternative, because it is a therapeutic method considered safe since the formulations are based on plants or minerals (Braghieri et al., 2007). Therefore, the possibilities of applying homeopathy in veterinary medicine are ample, opening a perspective for reduction of the indiscriminate use of conventional drugs, and consequently diminishing the selection pressure for development of resistant tick strains (Verdone, 2000; Veríssimo et al., 2016).

Thus, because of the lack of products and technologies to enable expansion of organic production in tropical countries, investigation of the efficacy of homeopathic medicines is of great interest, to enable validation and transfer of technology. Due to questions about efficacy and controversial results of studies (Mathie et al., 2012), more investigations about homeopathy need to be conducted to give scientific support to their efficacy. Thus, the objective of this study was to evaluate the efficacy of a homeopathic medicine on the tick *R. (B.) microplus* in naturally infested dairy cows.

Materials and methods

Location and experimental animals

The experiment was conducted for 12 months in 2015–2016 at the experimental dairy farm of Embrapa Pecuária Sudeste (CPPSE), located in the municipality of São Carlos, São Paulo state, Brazil (22° 01' S. latitude and 47° 54' W longitude, altitude of 856 meters). The climate in the region is high-altitude tropical, with dry winters. The indices of rainfall, relative air humidity, temperature, solar radiation and wind, covering the 12-month experimental period, were obtained from the weather station of Embrapa Pecuária Sudeste.

The animals were allocated in two paddocks (both with ± 3.0 hectares) formed of *Brachiaria* spp. and *Cynodon* spp. (coast-cross grass). They were kept under continuous grazing as routinely used for dairy herds, and the pasture was already naturally infested. The animals were non-lactating cows ($n = 24$) of mixed breed (Holstein and Jersey x Holstein), with average age of five years, without chemical treatment for at least 90 days before the start of the experiment. The experiment was authorized by the Committee on Ethical Use of Animals of Embrapa Pecuária Sudeste (CPPSE/Protocol 02/2014).

Homeopathic medicine and its administration

A biotherapeutic compound of *Rhipicephalus (Boophilus) microplus* 30CH and Sulfur 30CH was formulated by a homeopathic pharmacy. It was prepared with 1 part of the medicine (*R. (B.) microplus* engorged females) and 99 parts of lactose (vehicle), followed by maceration on a Hahnemannian centesimal scale, as recommended by the *Farmacopeia Homeopática Brasileira* (2011).

This procedure was employed successively until the 30CH dilution was obtained, which was evaluated in this assay. This medicine was chosen after a visit by a veterinarian specialized in homeopathy and dairy production systems, to learn the genetic profile of the herd and management conditions of the animals.

A volume of 5 ml of the homeopathic formulation was added to 500 g of sugar and was homogenized and stored. This preparation was then added to 30 kg of mineral salt (used as vehicle) and mixed with concentrated feed for supply to the animals. The group treated by homeopathy received feed composed of 80% corn, 15% soybean, 4.92% mineral salt and 0.08% sugar associated with the homeopathic substance, at a rate of 2 kg/animal/day. The control group received the same feed mixture in the same proportion, but without the homeopathic medicine. The feed was supplied to the animals of both groups daily in the morning, distributed in a common trough to the entire group.

Evaluation of the homeopathic preparation in the field

To randomize the groups, three tick counts were performed in advance on the 24 animals (August 2015). The left sides of these animals were inspected individually to quantify the engorged females larger than 4.5 mm in length (Wharton and Utech, 1970). The animals were then distributed into two groups ($n = 12$) with similar average number of ticks, and were sprayed with Colosso® (to kill all the ticks). The animals were identified by numbered tags and the groups were distinguished by colored collars. The groups were allowed to graze in the two paddocks during the entire experimental period and the ticks were counted weekly to determine the number of engorged females (>4.5 mm; left side of the cows). These counts were multiplied by 2 to estimate the total number of engorged females on each animal. The intention was to evaluate the natural infestation of each group, as well as the reinfestation in the pasture over time. The body weight (BW) of each animal was determined monthly and the packed cell volume (PCV) was measured every two months, by drawing 1.5–3 ml of blood by puncture of the middle coccygeal vein into a tube containing the anticoagulant EDTA.

Evaluation of the reproductive performance of the engorged females

Ten engorged females (>4.5 mm) were collected by hand from each group, always between 8 and 10 a.m. After each collection, to compare the reproductive parameters the engorged females were taken to the laboratory, examined to remove specimens with morphological deformities, washed with running tap water and dried on paper towels. The weight of the engorged females from each group was measured and they were placed in Petri dishes and kept in a heated chamber ($\pm 27^\circ\text{C}$ and RH $>80\%$) for 18 consecutive days for oviposition. Afterward, the eggs were weighed and placed in adapted syringes, which in turn were placed in the heated chamber under the same temperature and humidity conditions for 15 days for larval hatching. The hatching percentage and reproductive efficiency index (REI) were calculated according to Drummond et al. (1973).

Morpho-histological analyses

Semi-engorged females ($n = 25$) from each group were collected every three months. The semi-engorged stage was selected because the ticks have less degeneration of the organs than in the engorged stage and also due to the high parasite efficiency in this stage, since the ticks remain attached to the host's skin for blood meals, causing cutaneous lesions and possibly transmitting pathogens. The specimens were collected manually, cleaned and placed in

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