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Original Article

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

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A R T I C L E I N F O

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A B S T R A C T

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.

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* 30 CH and Sulfur 30 CH 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 30 CH 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 (±27 °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 well-ventilated recipients, identified by group. Then they were sent to the Histology Laboratory of the Department of Biology, Biosciences Institute, of São Paulo State University (UNESP), Rio Claro Campus, where they were examined under a photomicroscope to detect possible morpho-histological alterations of the ovaries.

The specimens were kept in a refrigerator to kill them by thermal shock and then they were dissected in a phosphate-buffered saline solution (NaCl 7.5 g/l, Na_2HPO_4 2.38 g/l and KH_2PO_4 2.72 g/l). The ovaries were removed, fixed for 24 h in 4% paraformaldehyde, dihydrated in ethanol, embedded in Leica resin for 24 h and transferred to plastic forms filled with polymerized Leica resin. After polymerization, all the blocks were cut into sections with thickness of 3 µm, stained with hematoxylin and eosin and mounted on glass slides for observation with a Motic BA300 photomicroscope (Oliveira et al., 2008).

Statistical analysis

The data on body weight of the cows, packet cell volume and number of ticks, in a fully randomized design, were submitted to analysis of variance with the PROC MIXED routine (SAS, 2002/2010), considering the effects of group (H $n=12$ and C $n=12$), month (1–12), and group*month interaction, in a split-plot design with repeated measures (months) in time. The Tukey test was applied for multiple comparisons of the means, using the LSEMANS option, with significance of 5%. The data on number of ticks were transformed by the square root of $x+0.5$ for normalization of the residuals. The environmental variables (temperature, relative humidity, rainfall, wind speed and solar radiation) were analyzed by descriptive statistics during the months (1–12).

The data on tick weight, egg weight, hatching percentage and reproductive efficiency index (REI) were also submitted to analysis of variance with the PROC GLM routine (SAS, 2002/2010), considering in the model the effects of group, season of the year and group*season interaction. Again the Tukey test was used for multiple comparison of the means ($p \leq 0.05$).

Results

There were no significant differences between the control group and the treated group that received the homeopathic medicine for 12 consecutive months (Table 1) with respect to body weight (BW), packed cell volume (PCV) and average number of ticks (Figs. 1–3).

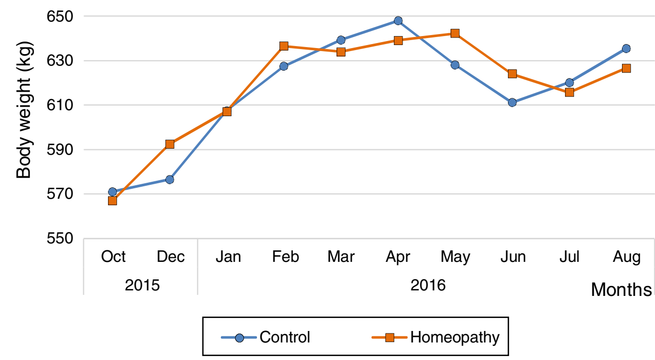

Fig. 1. Means of body weight of the animals, measured monthly, during the experimental period of 12 months.

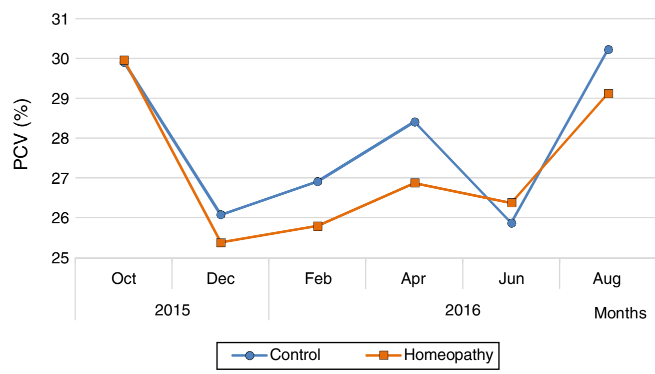

Fig. 2. Means of packed cell volume obtained from hemograms of the animals during the experimental period of 12 months.

Only eight animals needed to be treated with acaricide in June 2016, five belonging to the homeopathy group and three from the control group, by spraying with Colosso®, respecting a waiting period of 10 days before considering new counts or collection of ticks from these animals.

The rainfall was highest in December 2015 (66.6 mm), January 2016 (182.6 mm) and May 2016 (62.7 mm) (the summer and fall), while in April, July and August 2016 no rainfall was recorded. In the other months, the precipitation figures were: September 2015 (2.2 mm), October 2015 (0.2 mm), November 2015 (7.4 mm), February 2016 (16.8 mm), March 2016 (31.2 mm) and

Table 1
Means of body weight (BW) of cows, packed cell volume (PCV) and number of ticks on dairy cows of the control and homeopathic treatment groups (biotherapic de *Rhipicephalus (B.) microplus* 30 CH + Sulfur 30 CH), during 12 months, at the experimental farm of Embrapa Pecuária Sudeste.

Months	BW (kg)		PCV (%)		Ticks n°	
	C	H	C	H	C	H
2015						
Sept	[a]	[a]			35.88 ± 6.75	28.99 ± 6.63
Oct	571.1 ± 25.9	567.1 ± 26.0	30 ± 0.98	30 ± 0.97	30.33 ± 6.10	28.93 ± 5.92
Nov	[a]	[a]			4.31 ± 1.98	9.51 ± 1.91
Dec	576.7 ± 25.9	592.5 ± 26.0	26 ± 0.98	25 ± 1.00	2.01 ± 0.65	3.35 ± 0.64
2016						
Jan	607.5 ± 25.9	607.2 ± 26.0			3.64 ± 1.65	9.43 ± 1.60
Feb	627.6 ± 25.9	636.7 ± 25.9	27 ± 0.98	26 ± 0.97	20.21 ± 6.24	26.80 ± 6.02
Mar	639.4 ± 25.9	634.1 ± 25.9			16.13 ± 3.86	20.71 ± 3.79
Apr	648.1 ± 25.9	639.2 ± 25.9	28 ± 0.98	27 ± 0.97	4.42 ± 0.91	3.68 ± 0.90
May	628.2 ± 25.9	642.3 ± 25.9			10.48 ± 1.77	8.84 ± 1.74
June	611.2 ± 26.0	624.2 ± 25.9	26 ± 1.01	26 ± 0.97	13.62 ± 2.77	16.15 ± 2.68
July	620.2 ± 26.0	615.8 ± 25.9			4.06 ± 1.09	3.97 ± 1.07
Aug	635.5 ± 26.0	626.7 ± 25.9	30 ± 1.01	29 ± 0.97	1.24 ± 0.67	2.57 ± 0.65
Total mean	616.5 ± 25.4	618.6 ± 25.4	27.9 ± 0.72	27.3 ± 0.70	12.19 ± 2.27	13.58 ± 2.20

[a] Values not measured. There were no significant differences ($p \leq 0.05$).

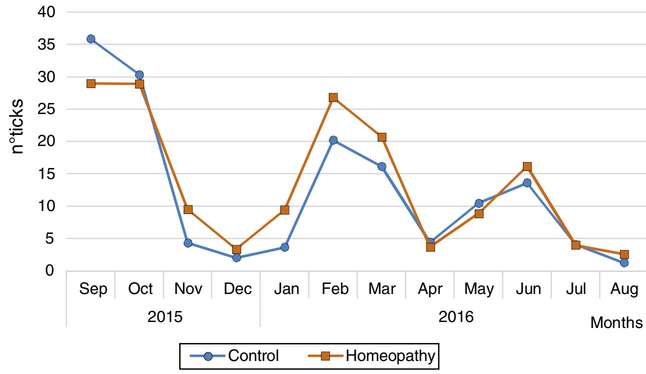

Fig. 3. Mean number of ticks counted on the cows' bodies during the experimental period of 12 months.

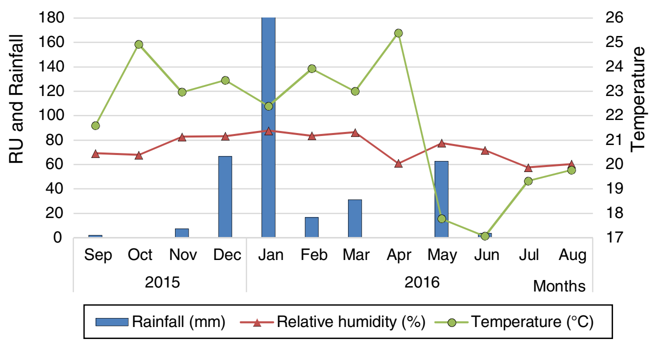

Fig. 4. Means of temperature, relative humidity and total rainfall, monitored by the weather station of Embrapa Pecuária Sudeste during the experimental period of 12 months.

June 2016 (3.8 mm). The average temperatures varied from 21.6 to 24.9 °C from September to November 2015 (spring), 22.4 to 23.9 °C between December 2015 and February 2016 (summer), 17.8 to 25.4 °C between March and May 2016 (fall), and 17.1 to 19.8 °C between June and August 2016 (winter). The relative humidity ranged from 67.8 to 82.8% in the spring, 83.3 to 87.7% in the summer, 61.1 to 86.4% in the fall and 57.5 to 71.9% in the winter (Fig. 4). The monthly average wind speed varied from 5.1 to 6.3 m/s in the spring, 5.9–7.9 m/s in the summer, 6.1–8.5 m/s in the fall, and 7.3–7.9 m/s in the winter. Finally, the solar radiation indices varied from 15.3 to 21.9 MJ.m-^{2} in the spring, 16.4–22.2 MJ m^{-2} in the summer, 12.8–20.7 MJ m^{-2} in the fall and 14.9–17.8 MJ m^{-2} in the winter.

The mean results of the *in vitro* comparison of the reproductive performance of the engorged females collected from both groups and incubated in the laboratory are reported in Table 2. No significant differences were observed ($p \leq 0.05$) for these parameters between the two groups.

Through application of morpho-histological techniques, it was possible to observe that the ovary of the individuals of the homeopathy group was composed of an epithelial cell wall and oocytes (germinative cells) in five development stages (I–V), connected to the wall by a peduncle. Fig. 5 depicts: (A) oocyte I, elliptical, with central germinal vesicle (nucleus) containing a clearly distinguishable nucleolus, homogeneous cytoplasm with absence granulations, surrounded by a thin cell membrane; (B) oocytes II, elliptical, with central germinal vesicle and fine and homogeneous cytoplasmic granulation; (C) oocyte III, rounded, germinal vesicle occupying the oocyte pole facing the peduncle and cytoplasm, filled with vitellum granules of various sizes, where the smaller granules occupied the central region and the larger ones the periphery; (D) oocyte IV, rounded, germinal vesicle rarely observed, but occupying the oocyte/peduncle pole, cytoplasm with many vitellum granules (various sizes randomly distributed), besides the start of chorion deposition; (E) oocyte V, rounded, germinal vesicle no longer observed and thick chorion (membrane) totally deposited. Besides this, small vacuoles were observed between the vitellum granules in oocytes II, III, IV and V. These results indicate no evidence of morpho-histological damage in the germinative cells (oocytes I, II, III, IV and V) of the females of the treated group. The semi-engorged females of both groups presented the same histological characteristics.

Discussion

In the present study we evaluated the use of a homeopathic medicine administered in the feed of cows naturally infested by *R. (B.) microplus*. The mean results of BW, PCV and tick number were not statistically different between the control and treated groups. Furthermore, no alteration in the ovaries was detected by microscopic analysis.

The 30 CH biotherapic formulation used in this study resulted in lower tick counts in the first two months, but the difference was not significant. Likewise, Valente et al. (2017) evaluated a 6 CH biotherapic formulation administered orally to artificially infested calves and obtained low efficacy after 42 days of treatment (10.13%). Although 30 CH (dilutions/dynamizations) are considered more potent than 6 CH, the results were not satisfactory. Low efficacy of homeopathic treatments to control ticks was also observed by Costa-Júnior and Furlong (2011). They evaluated the medicines Factor C&MC® (0.01 g/animal/day) and Sulfur-*Allium sativum*® (20 g/animal/day in feed) for five months in confined dairy cattle artificially infested with *R. (B.) microplus*, and observed efficacies of 26% and 64%, respectively. Different from our results, Müller and Fülber (2013) reported that the use of sulfur and *Staphysagria* was highly effective in herds, leaving the ticks pallid and flaccid, with altered color and easily removed with mechanical contact. Signoretti et al. (2013) also reported that engorged females from calves treated with Pró®, Estresse® and C&MC® factors (5 g/animal/day in 4 l of milk) had smaller size and abnormal dull coloration with yellow stripes on their backs.

This ineffectiveness of the homeopathic treatment might have occurred due to inadequate ingestion of the feed containing the homeopathic medicine, because it was distributed in a common trough to the entire group. When dairy cows are grouped, their social behavior changes, where they form hierarchies of dominance, especially in relation to access to food troughs (Friend and Polan, 1974). Therefore, the absence of locking yokes to separate the animals might have interfered in the quantity ingested by some animals. Also, although the animals were kept in separate paddocks, these were positioned alongside each other at a small distance. This could have interfered in the results because of movement of larvae between the paddocks. On the other hand, it is believed that homeopathic formulations do not act on isolated symptoms of a disease (considered an expression of disequilibrium), but rather by means of a process of releasing dynamic energy that will act in general in the organism, promoting a return to equilibrium of vital energy and consequently good health (Hahnemann, 1995). As a consequence, homeopathic therapy does not have directly curative properties. Instead, it provides conditions for the organism to combat adverse conditions (Verdone, 2000).

In our study, 41.6% of the animals in the homeopathy group were treated with an acaricide, a similar situation to the study by Signoretti et al. (2013), where 50% of the calves treated with Pró®, Estresse®, M&P® and C&MC® factors (5 g/animal/day in concentrated feed for 120 days) also received treatment with an acaricide. On the other hand, when the Pró®, Estresse®, M&P® and C&MC®

Table 2
Means of female tick weight (FW), egg weight (EW), % larval hatching (LH) and reproductive efficiency index (REI), obtained for engorged females of the control group (C) and homeopathic treatment group (H) during four seasons of the experimental period.

Data	Group[a]	Spring	Summer	Fall	Winter	Mean
FW (g)	C	0.21 ± 0.01	0.20 ± 0.02	0.19 ± 0.02	0.14 ± 0.02	0.18
	H	0.14 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	0.15 ± 0.02	0.17
EW (g)	C	0.10 ± 0.00	0.08 ± 0.01	0.07 ± 0.01	0.04 ± 0.01	0.07
	H	0.06 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.06
LH (%)	C	92.43 ± 4.91	84.07 ± 8.51	62.15 ± 9.91	41.86 ± 8.51	70.1
	H	87.70 ± 5.56	85.03 ± 8.90	74.70 ± 10.71	59.04 ± 10.71	76.6
REI	C	90.40 ± 5.67	65.31 ± 9.83	42.68 ± 11.44	18.99 ± 9.83	54.3
	H	72.40 ± 6.42	73.56 ± 10.28	56.61 ± 12.37	39.95 ± 12.37	60.6

[a] There were no significant differences ($p \leq 0.05$).

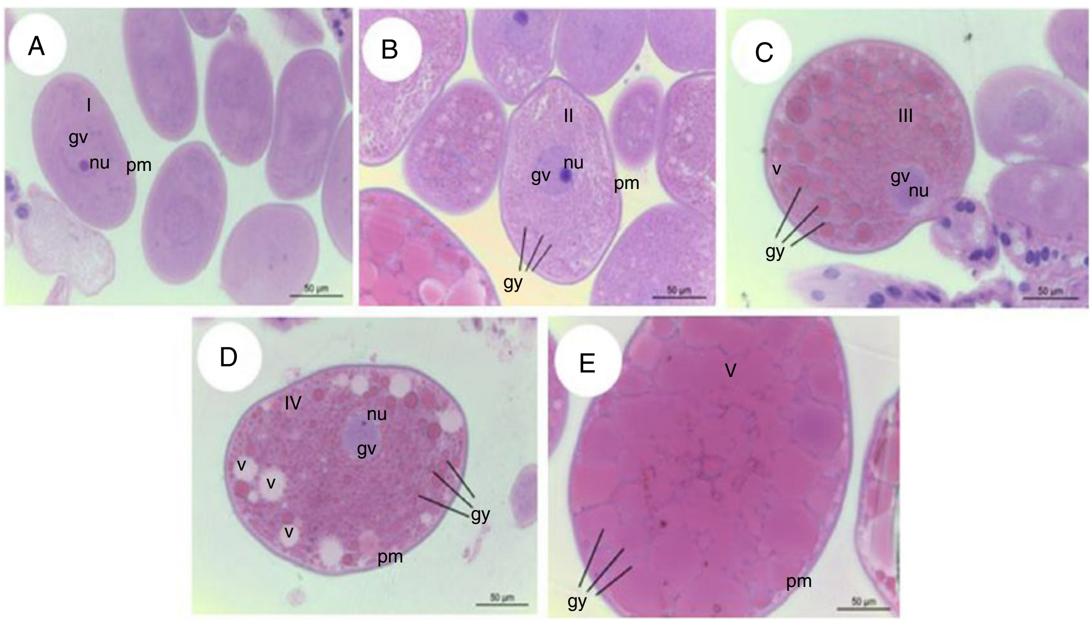

Fig. 5. (A)–(E) Histological sections of the ovaries of semi-engorged females of the tick *R. (B.) microplus* treated with the homeopathic medicine (biotherapic 30 CH + Sulfur 30 CH), demonstrating no alteration in the oocytes (stages I–V) stained with hematoxylin-eosin. nu, nucleolus; gv, germinal vesicle; v, vacuole; vitg, vitellum granules.

factors (5 g/animal/day in concentrated feed) were evaluated for 9 months in forty cows, no acaricide treatment was needed. A weight gain of 17.5% was also observed in the animals of the homeopathic group (Signoretti et al., 2010).

The homeopathic formulation did not have any effect on the weight between the groups ($p > 0.05$) in our study. Weight loss occurred in June 2016 due to the start of winter (when the pastures have lower availability of dry plant matter and lower forage nutritional value), and was also similar in both groups. However, Pinheiro et al. (2009) found lower body weight ($p < 0.05$) of the animals that received Factor C&MC® for 32 months (457.8 ± 30.7 kg) than those treated with an allopathic medicine (504.3 ± 39.2 kg). In the winter is season, we believe that the decline in weight, associated with the reduction of the hematocrit in June, demonstrates that the animals were under unfavorable physical conditions for immunological reaction to parasite infestation, which could have caused an increase in parasitism and the need for acaricide treatment in June.

In this study, the average tick counts were for the most part highest in the spring, followed by the summer, fall and winter. During the winter, the infestations tend to decrease due to the cooler temperatures and lower average relative humidity (RH), which impairs the free-living stage of the tick (Veríssimo and Machado, 1995; Kasai et al., 2000). The tick counts were lowest in April (RH 61%), July (RH 57%) and August 2016 (RH 60%). Very low RH can cause desiccation and infertility of eggs as well as interfere in hatching and survival of larvae, irrespective of the temperature. RH below 63% is responsible for lower hatching and larval survival (Davey et al., 1991).

Here, the peak infestation happened in the spring, while in the study of Veríssimo et al. (1997) it was observed in the fall. This difference can be associated with the low rainfall in springtime, and it seems that the higher infestation affected the PCV values in the beginning of the summer. The general trend is that the higher the infestation, the worse the weight gain and general conditions of the animal were, usually reflected by lower PCV. The monthly rainfall indices in the summer and fall, except for April 2016, were greater than 15 mm, tending to keep the tick populations low due to the movement and dispersion of the larvae, hampering their repositioning in places to wait for hosts (Rawlins, 1979). In the present study, the maximum wind speed (8.5 m/s) was recorded in the fall, and solar radiation *versus* reduction of parasites was not inversely proportional during the experimental period. Only winds stronger than 30.4 m/s are able to disperse tick larvae from their strategic points on the grass blades (Lewis, 1968), causing them to expend energy for repositioning and reducing their longevity (Wilkinson and Wilson, 1959; Furlong et al., 2002).

The differences in the reproductive parameters of the engorged females evaluated *in vitro* in the present study were not statistically significant. In contrast, Gazim et al. (2010) reported significant differences after treatment with two homeopathic preparations in mineral salt (12 CH daily for six months followed by 30 CH on alternate days until 28 months). The weight, egg mass and reproductive efficiency of the engorged females, as well as the hatching rates, were lower in relation to the control group. On the other hand, our results make sense, since no morphological or histological alterations were observed in the germinative cells of the females subjected to the homeopathic treatment. Hence, this indicates that it did not affect the reproductive parameters of the incubated engorged females. Valente et al. (2017) observed disorganization of the deposition of the *R. (B.) microplus* females' oocyte exochorion after treatment with a biotherapy, but it did not affect the efficacy against ticks (10.13%).

Lignon and Bottecchia (2005) reported that the adoption of homeopathic medicines to control diseases in cattle herds caused a reduction of 90% in the cost of veterinary drugs, improved the animals' behavior and increased the quality of milk, but this was only achieved in combination with other agro-ecological practices. Organic cattle breeding requires large modifications in management, normally accompanied by better nutrition. However, it is difficult to separate the effects of homeopathic medicines from those resulting from alterations in the system, especially related to the nutritional level (Costa-Júnior and Furlong, 2011). Therefore, perhaps the inclusion of agro-ecological practices would be necessary to obtain a proper evaluation of homeopathic medicines, because according to Lignon and Bottecchia (2005), even though homeopathy can be applied in conventional farms, it is necessary to adopt agro-ecological practices, such a reduction of artificial inputs, increase or maintenance of soil fertility with local resources, and introduction of other livestock species or various crops. These procedures can optimize the recycling of nutrients and improve the activity of the trophic chain by increasing biodiversity.

Conclusions

The evaluation of the biotherapic *R. (B.) microplus* 30 CH + Sulfur 30 CH to control natural infestations of *R. (B.) microplus* was not effective during the 12 months of this study, during which the infestation of the animals was only influenced by the climate parameters. There were no significant effects on the reproductive parameters of the engorged females and the morpho-histological traits of their ovaries. However, these conclusions cannot be extended to other homeopathic formulations, administration routes and management, since they were investigated under different conditions than in this study.

Authors' contributions

ACSC designed this research. AF, RRF, ICA, LGL, TCA and ACSC conducted the experiments. MICM and PRO contributed with morpho-histological analysis. WBJ analyzed data. AF and ACSC wrote the manuscript. All authors read and approved the manuscript.

Ethical disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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