Original article

Isotherapic for the control of the cattle tick

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ABSTRACT

Parasitism constitutes one of the major problems that affect the livestock. Commercial acaricides can propitiate drug resistance and residues in the environment and animals. This study aimed to evaluate the control of cattle ticks by using the isotherapics. These drugs were prepared using Rhipicephalus (Boophilus) microplus. The dilution and agitations were performed according to the homeopathic pharmacopoeia. By the immersion technique of adults, 50 teleogines were distributed into five treatments: 12cH and 32cH isotherapic, negative control, positive (ivermectin 1%) and vehicle control. The results were evaluated by the rate of mortality, egg production, hatchability rate, estimated reproduction, and the efficacy of the products. In addition, 16 Jersey females cows were subjected to 15mL pour on, of the following treatments: 12cH and 32cH isotherapic and vehicle control. The count of ticks was performed by the simple method on days 0, 15, 21, 42, 63, 84 and 105, post treatments. It was used the Wilcoxon test and Bonferroni method. Both isotherapics treatments showed efficacy at in vitro tests. The 12cH medication presented better results: 10% of mortality rate on the first day of life, maintaining 20% from the second to the 13th day. Additionally, it was observed 20% of egg production, hatching rate of 0.0038% and 99.39% of product effectiveness. However, the in vivo tests showed no satisfactory results for these evaluated conditions, although it was verified a tendency to decreasing the infestation.

Keywords: Agroecology. Organic production. *Rhipicephalus (Boophilus) microplus.*

Introduction

The Brazilian livestock has expanded in the last years [1], however, the bovine ticks are one of the major problems that affect this activity [2].

Chemical acaricides represent the main way of control; however the invisibility of the risks, associated with the management of pesticides for veterinary use, increases their exposure for producer and for consumer. The neglect of procedures is observed in the applying moment and in the grace period for human consumption, determining health problems [3]. Additionally, the frequent exposure to chemicals products, determined pharmacological resistance [4], which create new needs for repetitive use, that further aggravated the problem of resistance [5, 6]. For all this, were determined the search for natural alternatives, with biocide potential, that ensure not only animal health, but the food safety [7].

Consumers in different countries require more natural and better quality foods [8]. This scenario has favored the market of organic products. Organic agriculture is practiced on 35 million hectares in 154 countries, and despite the global recession, countries like Portugal and France, presented growing rates higher than 15% [9].

However, these systems require that the food should be produced without chemical residues. Therefore, the use of extracts of medicinal herbs [10], phytotherapies and homeopathic therapies [11] have been encouraged in agriculture. Thus, in order to avoid adverse effects on animal welfare and productivity of these organic herds further effective treatments should be tested.

Homeopathy presents like the basic principle, the use of diluted and agitated drugs [12]. Isotherapics are produced specifically from diseased or pathological substances, being innocuous in chemical terms [3]. Although homeopathy represent an important adjuvant agroecological production (Coelho et al., 2009), the scarcity of information about its effectiveness, discourages deployment in conventional systems and determines desistance in early deployment [14]. So, this study aimed to assess the control of cattle ticks by using isotherapics.

Material and Methods

The production of the isotherapics was performed at the Laboratory of Essential Oils and Phytotherapics of the Agricultural Sciences Centre, Statement University of North of Paraná / Agricultural Sciences Center. It was used *Rhipicephalus (Boophilus) microplus* collected from four Jersey cows, in lactation, naturally infected. These animals were previously submitted to the veterinary clinical examination and no alteration was observed. They were not subjected to any kind of allopathic or homeopathic treatment at least in 90 days.

In order to prepare the matrix solution, the parasites were crushed and added to the solution of grain alcohol 70%, distilled water and glycerin. It was used 6.6 ml for each of these constituents. For each measure of weight of the parasite, they were added four measures of this mixture, the measures were weight for weight. This matrix solution was identified, packaged in amber bottle and stored in a dry, ventilated and light-protected local. The vial was shaken daily for 30 seconds, for 20 consecutive days. After this period it was preceded filtration by filter paper and subsequent stand time for 48 hours.

In an ambar bottle of 30mL, it was added 19.8mL of grain alcohol at 70%, distilled water and glycerin (1:1:1), and 0.2 mL of matrix solution. After this, it was started the dilutions at a proportion of one to 99 of solvent, agitating up to 100 times, obtaining the 1cH (one hundredth) high dilution. In order to obtain 2CH, a portion of the content of this vial was removed and transferred to a second vial. After that it was added in 99 parts of solvent. Successive agitations were performed followed by dilutions until reach the potencies of 12 and 32cH [12].

For the realization of the in vitro assessments, the engorged ticks were observed under a stereoscopic microscope. They were selected by the absence of morphological changes (evaluation of their gnatosomes) and its physical state. They were cleaned, washed in tap water, dried with an absorbent paper and weighed in an analytical balance (accurate to 0.001g). Additionally, it was measured the length of the tick to promote the homogeneity between individuals. After that, it was proceeded with the random distribution into five treatments: 12cH and 32cH isotherapic drugs, negative control (received no treatment), positive control (ivermectin 1%) and vehicle control (grain alcohol at 70%, distilled water and glycerin).

The engorged female ticks were submitted to 20 mL of each treatment, for 5 consecutive minutes, using the immersion technique of adults (ITA) as recommended by some authors [15]. After immersion, these engorged females were dried with absorbent paper and allocated in Petri dishes according to their respective treatments. They were incubated, during 15 consecutive days, in a germination chamber with photoperiod, type Biological Oxygen Demand (BOD), at 27 °C and relative humidity of 80%.

It was evaluated mortality rate, egg production rate, hatchability rate, estimated reproduction and the efficacy of these products. The mortality rate was evaluated daily by the touch response [16]. It was evaluated the egg-laying period, weight of the egg mass and egg production rate (EPR) according to the formula:

$$EPR = 100 * [\text{egg weight (grams)} / \text{Initial female weight (grams)}]$$

After this period, and in order to verify the percentage of hatchability of the eggs, these eggs were transferred to glass sterile vials, which was properly identified and sealed with cotton wool. After incubation during 25 consecutive days, in B.O.D. chamber, under the same conditions of humidity and temperature described before, were daily evaluated, the rate of hatchability of eggs.

By the use of a stereoscopic microscope, the hatching of the eggs was evaluated by counting 10% of the larvae for each experimental group. The estimate of the total number of the larvae, was obtained by an adapted technique [17].

The product efficiency (PE) was determined according the calculation of the estimated reproduction (ER) [15] using the following formulas:

$$ER = [\text{egg weight (g)} \times \text{outbreak (\%)} / \text{Female mass (g)}] \times 20{,}000$$

where the factor 20,000 is a constant that indicates the number of eggs present in 1g of egg-laying.

$$PE = 100 \times (\text{ER control} - \text{ER treated}) / \text{ER control}$$

The acceptable efficiency for a chemical acaricide base to be licensed in Brazil, must be equal or greater than 95%, on a sensitive strain of *Rhipicephalus (Boophilus) microplus* [18, 19]. Thus, the results were interpreted considering as effective the minimum value of 95%. The Wilcoxon test [20] was used to compare the median percentage of dead ticks between treatments.

To perform the in vivo study, 16 lactating Jersey cows were distributed in three treatments: 12cH, 32cH isotherapic drugs and vehicle control. The treatments were performed using equal volume of 15mL by pour on as way of administration. The results were assessed before and after the use of the treatments, at 15, 21, 42, 63, 84 and 105 days. These results were obtained by a simplified counting method [21]. It was considered engorged female ticks with presenting at least 4 mm, in the following regions: head, neck, chest, axilla, scapular region and forelimb. For statistical analysis, it was used Wilcoxon test [20], in order to compare the median of the variables for the treatments, and for the evaluated periods. To the Bonferroni method [21] was necessary to adjust the P-value resulting from multiple comparisons.

This research was submitted to the Ethics Committee on Animal of the State University of North of Parana, ensuring that it complies with ethical principles of animal experiments present in Brazilian legislation.

Results

The analysis related to the length (Table 1) and weight (Table 2) of the ticks in the moments before the use of the treatments, showed similarity between the individuals tested. In relation to the weight of engorged females, there was a mean of 1.54 grams.

Table 1 - Median and standard deviation of the length, in millimeters, of the engorged female ticks for the treatments: 12cH and 32cH isotherapics, negative control and vehicle control.

12cH isotherapic	32cH isotherapic	Vehicle Control	Positive Control	Negative Control
7.0 $\pm$1.0	5.8 $\pm$ 1.0	6.0 $\pm$ 0.9	8.0 $\pm$ 0.6	6.0 $\pm$ 0.7

Table 2 – Median and standard deviation of the weight of engorged female ticks, in grams, in the moment before the use of treatments, weight of eggs in grams, egg production rate (EPR), hatching of egg percentage, estimated reproduction (ER) and effectiveness of the product (EP) for treatments: 12cH and 32cH isotherapics, negative control, positive control and vehicle control.

Treatment	Median of Gravid Female Ticks Weight (g)	Median of Eggs Weight (g)	EPR%	Eggs Hatching %	ER	EP
12cH isotherapic	1.45	0.29	20	0.038	152	99.39
32cH isotherapic	1.57	0.61	38.85	0.63	4896	98.06
Vehicle Control	1.54	0.57	37.01	27.92	206680	18.22
Positive Control	1.61	0	0	0	0	100
Negative Control	1.48	0.61	41.21	30.66	252738	0
Median/ standard deviation	1.54 ± 0.06	0.57± 0.26				

Regarding the mortality rate, after 12 hours after the immersion test, it was recorded 100% of mortality for the engorged female ticks submitted to the positive control treatment.

The animals submitted to the 12cH isotherapic treatment showed 10% of death on the first day, and the value of 20% was maintained until13th day. The 32cH isotherapic presented 10% of mortality only after the 11th day. The negative control and vehicle control groups showed low mortality (10%) only from the 9th and 13th day, respectively. From the 13th day of evaluation, all the ticks began to present the morbid process, independent of the treatment that was used. Isotherapics groups of the 12cH and 32cH showed adequate efficacy, with 99.39 and 98.06%, respectively. The results about the egg production rate (EPR), hatchability, estimated reproduction and efficacy of the product are shown in Table 2.

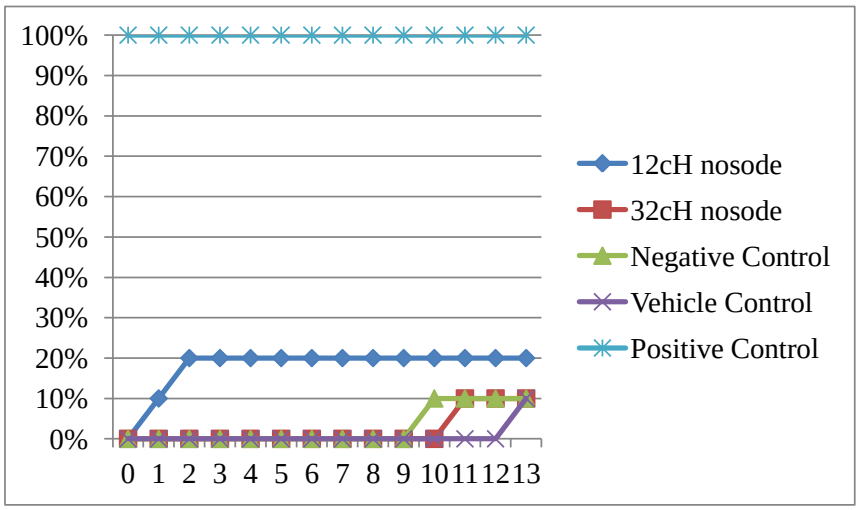

Figure 1 - Mortality rate, in percentage, of the engorged female ticks for the treatments: 12cH and 32cH isotherapics (nosodes), negative control and vehicle control, after 13 days of the use of their treatments.

For the conditions of this study, the using in vivo of both isotherapics treatments (12cH and 32cH) was not able to control the tick infestation (Table 3). Values higher than 25 ticks indicate the necessity for a therapeutic intervention [21].

Table 3 – Average distribution of the number of ticks, *Rhipicephalus (Boophilus) microplus,* according to the simplified counting method, in the animals from 12Ch and 32Ch isotherapic treatment, and negative control (NC); moment immediately before the treatments (0) and after, on 15, 21, 42, 63, 84 and 105 days. (*) Necessity to interrupt the isotherapic treatment.

Treatments	Days of evaluation of the *in vivo* tests					
	0	21	42	63	84	105
12 cH	11	37.40	*	*	*	*
Vehicle Control	26	34.90	*	*	*	*
32 cH	21.30	56.50	13.80	34	14	18.80
Vehicle Control	15.30	34.80	9.75	22	18.50	38.50

Discussion

Considering the Ministry of Agriculture, Livestock, and Supply [18] preliminary results relating to the in vitro tests showed that both isotherapics treatments were considered effective. The efficacy of isotherapics treatments was demonstrated by the reproduction of the engorged female ticks, since these treatments showed lower production of eggs when compared to the eggs production of the groups: negative control and vehicle control.

Assuming that different diluents commonly used in the composition of vehicles for the constitution of commercial products, may influence the biology of ticks and thus interfere with the results presented by adults immersion test [15], the present study evaluated beyond the negative control group, a group constituted of an oily solution vehicle. Corroborating with these findings [15], this study also found interference of the oily vehicle on the biology of ticks. These researchers [15] evaluated the efficacy of 30 commercial insecticides on two species of ticks (Boophilus annulatus (Say) and Boophilus microplus (Canestrini) using the adult immersion test. They found that their data for larvae tested with water-based formulations were favorable but not for larvae tested with oil-based solutions.

For the short period of five minutes used at the adults immersion test, the water solutions does not interfer at the ticks [22]. These researchers evaluated the effect of immersion in distilled water on the reproductive parameters of engorged females of Rhipicephalus (Boophilus) microplus. They evaluated long periods of immersion, ranging from two to 120 hours. They found that over a period of up to 30h of immersion, the engorged females performed oviposition, although more than 50% of eggs failed to hatch.

Concerning the in vivo experiment, the dosage of 12cH was not be able to provide a minimum condition of animal welfare in the first moment of evaluation. Similarly, other researchers [23, 24] did not observed positive results when evaluating isotherapics at 12cH, even after 28 months of observations. Thus there was a need to evaluate higher dose, so we tested 32cH. However it still has not been possible promote the control of ticks, but it was verified statistical tendence to reduce the ticks infestation. Nevertheless, in contradiction to the results that we found, another research evaluating isotherapic at the power output of 32CH, was able to register decrease of the number of engorged female ticks and reduced of the hatching rate of larvae. Worth noting that such authors performed their studies in temperate region, which has the best conditions for the

control of ticks. However, the same does not occur for the conditions of this study, since the conditions of temperature and humidity of the tropical Brazilian climate can promote important stimulus for proliferation of ticks [25].

Conclusion

From the results obtained by the in vitro evaluations for the isotherapic drugs 12 and 32cH the treatments showed efficacy for the control of cattle ticks. Regarding the in vivo experiment, although there has been no significant effect, we could verify a tendency to reduction of the infestation in these animals.

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