

Efficiency of tick biotherapeutic on the control of infestation by *Rhipicephalus (Boophilus) microplus* in Dutch dairy cows

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ABSTRACT

Background: cattle tick *Rhipicephalus (Boophilus) microplus* poses serious problems for farmers in Brazil, especially because the parasite easily develops resistance to pesticide agents. For this reason, together with other factors including environmental, human and animal contamination and costs, alternative approaches have been sought for. **Aims:** this study sought to evaluate the efficiency of a tick biotherapeutic on tick-infested cows. **Methods:** 34 dairy Dutch cows were divided in 2 groups: one group received 100g/day of mineral salt supplement impregnated with tick biotherapeutic 12cH for 6 months, and then in alternate days with tick biotherapeutic 30cH to complete 28 months of treatment; the other group (control) received only the mineral salt supplement. After 28 months of treatment, engorged *Rhipicephalus (boophilus) microplus* females were collected in both groups, counted and weighed; in vitro tests were carried out to assess mass of ticks; egg mass; egg-hatching rate; and reproductive efficiency. **Results:** There was significant difference between both groups for all parameters evaluated; tick-mass ($p = 0.0008$); egg mass ($p=0.0044$); egg-hatching rate ($p= 0.0017$); and reproductive efficiency ($p = 0.0044$). **Conclusion:** treatment with tick biotherapeutic significantly decreased the mass of engorged females, deposition and hatching rate of eggs, resulting consequently in the decrease of the reproductive efficiency of ticks.

Keywords: Homeopathy; *Rhipicephalus microplus*; Tick biotherapeutic; Controlled trial; Cows

Introduction

Tick *Rhipicephalus (Boophilus)* is extremely specific to cattle however it can also sporadically parasitize other species of animals [1]. As a mostly tropical country, the climate of Brazil favors the growth and survival of *R. microplus* [2].

This ectoparasite carries protozoa (*Babesia bovis* e *Babesia bigemina*) and prokaryotes (*Anaplasma marginale*), which cause babesiosis and anaplasmosis respectively, affecting severely the production of meat and milk in farms, as well as restricts the transit of animals [3]. Damage can be direct and indirect: the most significant causes of direct damage are the annoyance of animals by stings; the depletion of bodily nutrients

due to the tick's sucking of blood and lymph; and the toxic effect of stings. Indirect damage includes tick-borne diseases and the expenses related to tick and disease control [4].

In the life cycle of *R. microplus*, only one host is needed. Mating of ticks usually occurs while they are on the host animal; afterwards, the female drops to the ground and after 2-6 days it begins to deposit eggs (about 3,000) that remain agglutinated. Oviposition lasts 15-20 days to finish when the females dies. The period of incubation lasts 6-7 days and depends on the environmental temperature and the relative humidity of the air. Freshly hatched larvae do not have infesting ability, but after 4-6 days they climb the stems of grass and wait for hosts to pass by [5].

As a rule, control of *R. microplus* in Brazil is grounded on bathing animals with pesticides once ticks are identified. The number of such baths can be very high varying with the method of application, time of the year, race of animals and cost of treatment [2]. However, the ability of this parasite to develop resistance to pesticides restricts their use and farmers tend to increase both the dose and the frequency of application [1]. Resistance of *R. microplus* to pesticides increases anywhere it finds favorable conditions to development; in Brazil, it was identified several populations resistant to different pesticides [6].

The main phenomena associated with resistance to pesticides are: alteration of the external tegument, decreasing the rate of penetration of drugs; metabolic changes leading to the storage and excretion of drugs; and dislocation of the target-action of drugs. Most pesticides are not sufficiently effective so that resistant ticks survive and activate their enzymatic defense system, transmitting to the offspring the resistance thus acquired [7].

Misuse of conventional pesticide agents together with the resistance against them developed by ticks, human and environmental contamination and the presence of residues in the marketed products add further problems related to these ectoparasites [8]. The indiscriminate use of chemicals affects the environment, animals and people, on the other hand, the use of natural products can minimize the ecological imbalance and the pollution of the environment [1]. Furthermore, since resistance to anti-parasite drugs develops so easily, the pharmaceutical industry hesitates to invest in developing new drugs. Although the time required to market a new drug is hard to estimate, it is certainly limited due to the fast development of resistance [9].

Control of endo- and ectoparasites through homeopathy and/or isopathy has been attempted several times in Brazil with good results [7]. The reasons adduced by producers to seek alternative approaches include: high cost of conventional treatment; emergence of drug resistance; human and environmental contamination; elimination of natural enemies; interest in entering the organic products market [10]. Veterinarians highlight 4 features to explain the growing use of homeopathy by farmers: effective results; lack of potential drug resistance; lack of sediment in food; and lower cost. A further advantage is that there is no need to observe a time-gap between the use of medicines and the trade of food-producing animals, decreasing dramatically financial losses [11].

For these reasons, the aim of the present study was to test a biotherapeutic preparation of *R. microplus* on dairy cattle to verify its potential efficacy in the control of this ectoparasite.

Materials and Methods

Biotherapeutic was prepared at the Pharmacotechnics and Homeopathy Laboratory of Universidade Paranaense (Unipar), Brazil, from engorged *R. microplus* females according to the procedures described by Brazilian Homeopathic Pharmacopeia (FHB) [12]. Live ticks were placed in contact with a liquid extractor containing water, ethanol and glycerin in proportion 1:1:1 for 20 days under agitation. The resulting solution was

filtered, diluted and agitated until reaching dilution 12cH. This dilution was used to impregnate mineral salt in proportion 10% according to the prescription for impregnation of powders in FHB; the compound was then dried in heaters at less than 50°C. Mineral salt was chosen since it is a daily supplement for cattle.

The study was carried out at farm São Tomé, Umuarama, Paraná, Brazil. Thirty-four dairy Dutch cows were divided in 2 groups: Group I (n=17) received plain mineral salt (negative control) and Group II (n=17) received mineral salt impregnated with tick biotherapeutic 12cH (treated group). The batch of mineral salt was the same for both groups and the dose was 100 mg/day. Both groups were kept in the same area but in different pastures to facilitate handling.

The study began in 27 January 2007. After 6 months, the dilution of tick biotherapeutic was changed to 30cH and the dose was administered on alternative days instead of daily.

After 28 months of treatment, on 29 March 2009 the engorged females of tick *R. (B.) microplus* were collected in both groups; selection criterion was maximal engorgement. Females were cleansed and evaluated regarding their integrity at maximum engorgement; 46 females were selected among the ones collected from the animals treated with biotherapeutic and 30 from the control group. They were transported to the Parasitology Laboratory of Unipar where it was carried out the *in vitro* tests described by Drumond [13].

The selected engorged females were individually weighed and identified before being distributed in 9-cm Petri dishes containing 10 ticks each. The experiment was repeated 3 times, totaling 30 females per group. The Petri dishes were incubated at 28°C for 14 days until oviposition; it was measured the egg-mass of each individual female. Then the eggs were placed in test-tubes kept at 27-28°C and 70-80% relative humidity for 21 days until eggs hatched.

Results

The parameters evaluated in this study through *in vitro* tests included: mass of ticks; egg mass; egg-hatching rate; and reproductive efficiency in both groups. It was observed homogeneity among the females chosen regarding their weight between both groups according to variance analysis. This parameter is crucial to establish the egg-hatching rate, since a poor distribution of weight can mask its results.

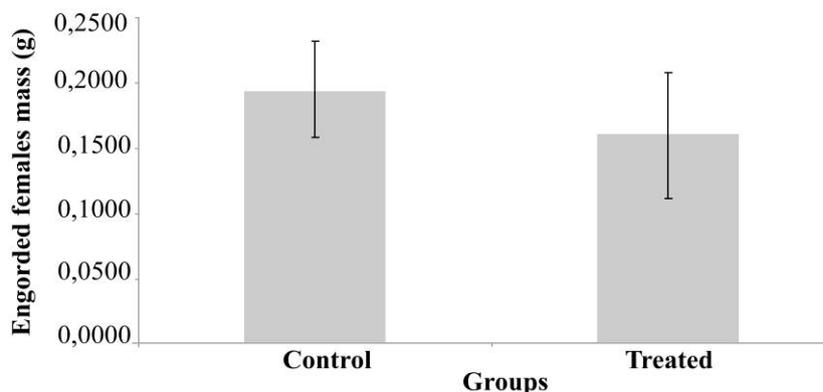


Figure 1: Mass of engorged females in the group treated with *R. microplus* biotherapeutic 12cH and 30cH and in the control group (t-test p=0.0008).

Figure 1 depicts the results of tick mass in both groups. There was significant difference in the ticks mass between the treated and control groups ($p = 0.0008$), which means that the females that parasitized the treated group of cows had a lower mass gain than the ones that parasitized the control group.

Fourteen days after the ticks were placed in plates, the egg mass was measured; results are shown in Figure 2; there was significant difference between the treated and control groups.

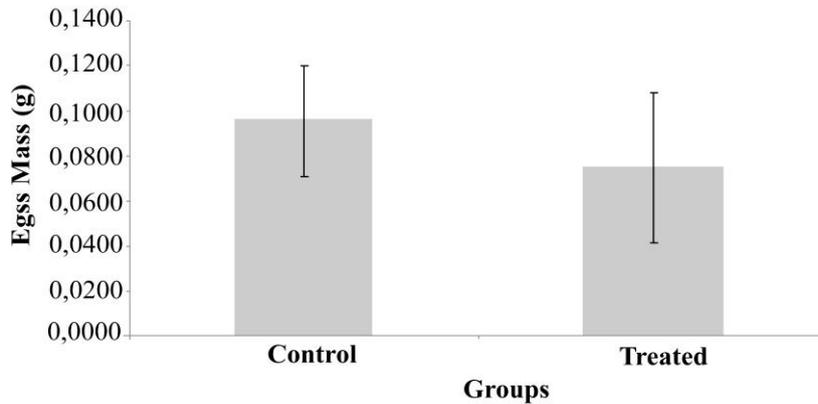


Figure 2: Egg mass of the group treated with *R. microplus* biotherapeutic 12cH and 30cH and of the control group (t-test $p=0.0044$).

Figure 3 represents the percentage of egg-hatching in the treated and control groups; there is significant difference between both groups, showing a lower egg-hatching rate in the treated group.

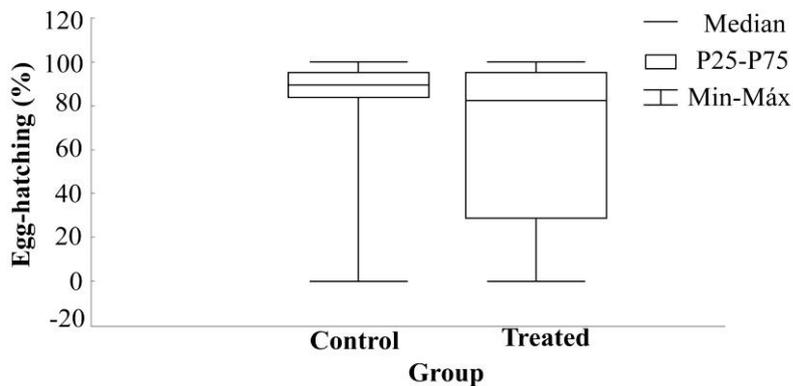


Figure 3: Percentage of egg-hatching in the group treated with *R.(B.) microplus* biotherapeutic 12cH and 30cH and in the control group (t-test $p= 0.0017$).

The results of reproductive efficiency are represented in Figure 4.

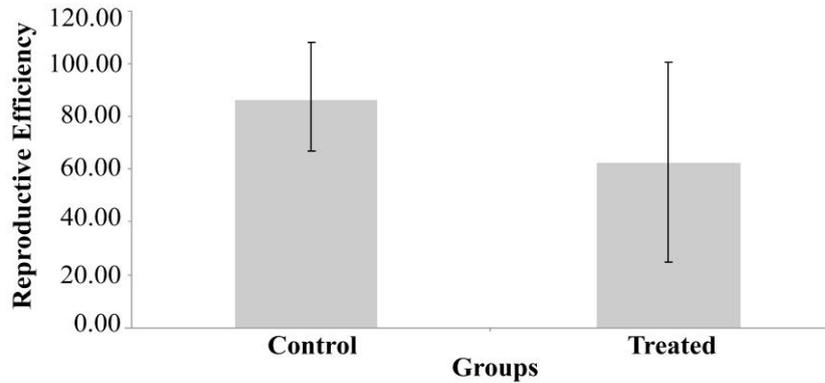


Figure 4: Reproductive efficiency of the group treated with *R.(B.) microplus* biotherapeutic 12cH and 30 cH and the control group. (t-test $p=0.0044$).

RE was estimated according to equation 1, taking into account the weight of engorged females and eggs, the egg-hatching percentage and the approximate number of larvae per gram of eggs ($\approx 20,000$). Results show significantly lower RE in the tick treated than in the control group ($p = 0.0044$).

$$ER = \frac{\text{eggs weight}}{\text{engorged females weight}} \times (\% \text{egg hatching}) \times (20,000) \quad (\text{eq. 1})$$

Discussion

After 3 months of treatment, it was observed a decrease in the number and size of tick females in the cows treated with biotherapeutic. This allowed the farmer to decrease the frequency of pesticide baths, which was every 15 days before the study began, to every 2 months and then at gradually longer intervals, until at the end of the study no baths whatsoever were further needed.

Regarding the decrease in tick infestation, our results can be compared with the ones obtained by Neto [15], who also used homeopathic preparations mixed to a mineral supplement and observed a lowering of the infestation by ticks in the treated animals.

This decrease in tick infestation was also shown in the studies by Real [16], evaluating the control of ticks through population-homeopathy. This effect allowed widening the intervals between the administration of pesticides and insecticides resulting in a overall reduction in their use as long as homeopathic treatment was continued.

The significant difference of the egg mass between the treated and control groups can be explained as a consequence of the weight of females being significantly lower in the treated group than in the control group.

The *in vitro* tests still demonstrated the efficacy of the tick biotherapeutic on the egg-hatching rate, which was 50% (30-95%) in the treated group and 85-95% in the control group. This effect can also be explained as an effect of the reduction in the weight of females, with the corresponding decrease in egg mass and consequently, in the rate of egg hatching.

The experiment still included the estimation of the Reproductive Efficiency, which was about 22% lower in the treated group compared to the control.

These results agree with the ones obtained by Verissimo [17], who used tick biotherapeutic in dilution 6cH and observed decrease in tick infestation in the animals included in the study. This decrease of the tick population was also observed by Arenalis [18], in whose study, after 7-10 days of ingested the blood of animals treated with homeopathic preparations, the ticks exhibited difficulty to feed and began to wither until acquiring the aspect of mummification. In Almeida's revision [19], some studies showed that the administrations of ultra diluted antigens is able to influence the immune modulating response; this may help to explain the effect of tick biotherapeutic on the life cycle of the parasite. Silva [20] used a biotherapeutic mixture on 27 Purunã heifers and showed significant effect on the control of infestation by *R. microplus*, with 50% reduction in the number of engorged females.

The use of dilutions 12cH and 30cH dilution is justified by Neto [15], who explains that concentrations 10^9 - 10^{23} correspond to the concentrations of hormones and neurotransmitters in the body.

Alternatives to tick control by using phytotherapy are shown by Farias [21], who evaluated the *in vitro* action of *Carapa guianensis* Aubl (andiroba) seed oil and obtained 100% efficacy in the mortality and egg production of engorged *B. microplus* females. Borges [22] used hexane extracts of ripen fruits of *Melia azedarach* L. and observed an interference on the development of ticks on animals but not on their reproduction. On the other hand, Silva [23] observed a reduction in the reproductive parameters of ticks using *Azadirachta indica* and *Cymbopogon citratus*, probably due to interference on oviposition.

Further relevant data were supplied by Chagas [1], who tested diverse solvents on engorged females and measured egg deposition and hatching rate. The results allowed concluding that the liposoluble and vehicles solvents (alcohol, acetone) have the highest toxic effect on engorged females. These data ought to be taken into account when attempting to develop new pesticide drugs.

Conclusion

There was significant interference in the life cycle of ticks recovered from cows treated with tick biotherapeutic 12cH and 30cH. *In vitro* tests showed mass reduction of engorged females, oviposition reduction, egg-hatching rate and reproductive efficiency.

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Eficiência de bioterápico de carrapato no controle da infestação por *rhipicephalus (boophilus) microplus* em vacas leiteiras de raça holandesa

RESUMO

Introdução: os carrapatos do gado *Rhipicephalus (Boophilus) microplus* representam um sério problema para os criadores brasileiros, especialmente porque desenvolvem rapidamente resistência aos pesticidas. Por isso, junto a outros fatores incluindo contaminação ambiental, humana e animal, assim como os custos, têm sido procuradas abordagens alternativas. **Objetivos:** avaliar a eficiência de um bioterápico desenvolvido a partir de carrapatos em vacas infestadas. **Métodos:** 34 vacas leiteiras de raça holandesa foram divididas em 2 grupos; um recebeu 100 mg/dia de suplemento salino mineral impregnado com o bioterápico 12cH durante 6 meses e após 30cH em dias alternos até completar 28 meses de tratamento; o outro grupo (controle) recebeu só o suplemento mineral. Após 28 meses de tratamento foram coletadas fêmeas ingurgitadas do carrapato em ambos os grupos, contadas e pesadas, e foram realizados testes *in vitro* para determinar a massa de parasitas; massa de ovos; taxa de eclosão dos ovos; e eficiência reprodutiva. **Resultados:** houve diferença significativa entre ambos os grupos em todos os parâmetros avaliados – massa de parasitas ($p=0,0008$); massa de ovos ($p=0,0044$); taxa de eclosão de ovos ($p=0,0017$) e eficácia reprodutiva (0,0044). **Conclusão:** o tratamento com bioterápico de carrapato diminuiu significativamente a massa de fêmeas ingurgitadas e a deposição e taxa de eclosão, resultando, conseqüentemente, na diminuição da eficiência reprodutiva dos carrapatos.

Palavras-Chave: Homeopatia; *Rhipicephalus microplus*; Bioterápico de carrapato; Estudo controlado; Vacas

Eficiencia de bioterápico de garrapata para control de la infestación por *Rhipicephalus (Boophilus) microplus* en vacas lecheras de raza holandesa

RESUMEN

Introducción: la garrapata del ganado *Rhipicephalus (Boophilus) microplus* produce serios problemas a los hacendados brasileños especialmente porque desarrolla rápidamente resistencia a los agentes pesticidas. Por este motivo, asociado a otros factores como contaminación ambiental, humana y animal y los costos, se buscan abordajes alternativos. **Objetivos:** este estudio buscó evaluar la eficiencia de un bioterápico preparado de garrapatas en vacas infestadas. **Métodos:** 34 vacas lecheras de raza holandesa fueron divididas en 2 grupos; uno recibió 100 mg/día de suplemento mineral impregnado con bioterápico 12cH durante 6 meses y después, en días alternados hasta completar 28 meses de tratamiento; el otro recibió apenas el suplemento mineral (control). Después de 28 meses de tratamiento, garrapatas hembra ingurgitadas fueron recogidas en ambos grupos, contadas y pesadas; fueron realizados pruebas *in vitro* para medir la masa de garrapatas; la masa de huevos, la tasa de eclosión de huevos y la eficiencia reproductiva. **Resultados:** hubo diferencias significativas entre los 2 grupos en todos los parámetros evaluados – masa de garrapatas ($p=0,0008$), masa de huevos ($p=0,0044$), tasa de eclosión de huevos ($p=0,0017$) y eficiencia reproductiva ($p=0,0044$). **Conclusión:** el tratamiento con bioterápico de garrapata redujo significativamente la masa de hembras ingurgitadas y la deposición y eclosión de huevos, resultando en disminución de la eficacia reproductiva de las garrapatas.

Palabras-Llave: Homeopatía; *Rhipicephalus microplus*; Bioterápico de garrapata; Ensayo controlado; Vacas



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