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Influence of *in vitro* simulated precipitation on acaricide treatments to control *Rhipicephalus microplus*

Influência da precipitação simulada *in vitro* em tratamentos acaricidas para controle de *Rhipicephalus microplus*

Fagner D'ambroso Fernandes[1]; Patrícia Bräunig[2]; Renata Rojas Guerra[3]; Luis Antônio Sangioni[3]; Fernanda Silveira Flores Vogel[3]

Highlights

Influence of precipitation on acaricide treatment.
Acaricide treatment with Colosso FC30 to control *Rhipicephalus microplus.*
Acaricide treatment with Colosso FC30 after precipitation.

Abstract

Rhipicephalus microplus is a widely distributed tick species that causes direct and indirect losses to cattle production; therefore, the control of tick infestations is essential. Correct treatment methods are necessary to avoid overdosing or underdosing of chemical acaricides and control tick resistance. Moreover, studies that highlight the factors associated with the efficacy of chemical products are extremely necessary. Therefore, this study aimed to evaluate the effects of precipitation intensity and time on the efficacy of topical application of acaricides. Engorged females were collected from cattle on farms located in Rio Grande do Sul, Brazil and treated with Colosso FC30® Ouro Fino Animal Health (15% cypermethrin, 30% chlorpyrifos, and 15% fenthion). After 30 min, 1 h, 2 h, 3 h, 6 h, 9 h, 12 h, or 24 h of treatment, they were subjected to simulated precipitation at mild and intense, for each time. The results showed 100% acaricide efficacy for all the evaluated groups and demonstrated that precipitation from 30 min after treatment and at any intensity, did not affect the efficacy of Colosso FC30. Therefore, this study clarified the influence of simulated precipitation on the efficacy of topical Colosso FC30 treatment towards the *R. microplus* control.

Key words: Tick. Precipitation. Resistance. Efficacy. Colosso FC30.

[1] Prof. M.e, Veterinary Medicine, Course Centro Universitário Ritter dos Reis, UniRitter, Porto Alegre, RS, Brazil. E-mail: fagner.fernandes@acad.ufsm.br

[2] Drª, Universidade Federal de Santa Maria, UFSM, Santa Maria, RS, Brazil. E-mail: pbraunig@gmail.com

[3] Profs. Drs., UFSM, Santa Maria, RS, Brazil. E-mail: renata.r.guerra@ufsm.br; luis.sangioni@ufsm.br; fernanda.vogel@ufsm.br

* Author for correspondence

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Resumo

Rhipicephalus microplus é uma espécie de carrapato amplamente distribuída que causa perdas diretas e indiretas à produção de gado; portanto, o controle de infestações de carrapatos é essencial. Métodos de tratamento corretos são necessários para evitar sobredosagem ou subdosagem de acaricidas químicos e controlar a resistência aos carrapatos. Além disso, estudos que evidenciem os fatores associados à eficácia dos produtos químicos são extremamente necessários. Portanto, este estudo teve como objetivo avaliar os efeitos da intensidade e do tempo de precipitação sobre a eficácia da aplicação tópica de acaricidas. Fêmeas ingurgitadas foram coletadas de bovinos em fazendas localizadas no Rio Grande do Sul, Brasil e tratadas com Colosso FC30® Ouro Fino Animal Health (15% cipermetrina, 30% clorpirifós, and 15% fention). Após 30 min, 1 h, 2 h, 3 h, 6 h, 9 h, 12 h ou 24 h de tratamento, foram submetidas à simulações de precipitação em intensidade leve ou intensa, para cada tempo. Os resultados mostraram eficácia acaricida de 100% para todos os grupos avaliados e demonstraram que a precipitação de 30 min após o tratamento e em qualquer intensidade, não afetou a eficácia do Colosso FC30. Portanto, este estudo esclareceu a influência da precipitação simulada na eficácia do tratamento tópico Colosso FC30 no controle de *R. microplus.*
Palavras-chave: Carrapatos. Precipitação. Resistência. Eficácia. Colosso FC30.

Rhipicephalus microplus is a widely distributed tick species that mainly infests cattle. Ticks may act as potential vectors for viruses, bacteria, and protozoa in animals and humans (De la Fuente et al., 2008; Jongejan & Uilenberg, 2004). Tick infestations may cause hemoparasitic infections, reduction in milk and meat production, leather depreciation, and the death of infested animals (Grisi et al., 2014).

Economic losses associated with tick infestation in cattle production are of great importance (Jonsson et al., 2001). The cost of acaricide treatments exceeds US $130 million per year in Brazil, and worldwide economic losses caused by tick infestation in cattle are US $6.86 billion per year (Grisi et al., 2014).

Sprinkling or immersion baths are topical treatments widely used for combating ticks in cattle; these treatments include several molecules from different chemical groups, with organophosphates, pyrethroids, and amidines being the principal molecules used (Meng & Sluder, 2018). However, inappropriate treatment involving inadequate concentrations or inappropriate combination of chemical products and incorrect frequency of treatment cause tick resistance (Food and Agriculture Organization of the United Nations [FAO], 2003; Klafke et al., 2017; Meng & Sluder, 2018).

In addition to resistance, humidity, temperature, and host susceptibility are the main factors that determine tick parasitism. Favorable climatic conditions improve tick parasitism, consequently increasing the cost of tick control (Jongejan & Uilenberg, 2004). The establishment of *R. microplus* larvae can be conditioned to changes being climatic variations, temperature and precipitation variations, as well as the result of human activities (Estrada-Pena, 2009).

As for rainfall intensity and tick epidemiology, the studies correlate rainfall intensities with oviposition and tick distribution, not considering the influence on the time of precipitation with the time when the acaricide treatment was performed (Adejinmi, 2011). In addition, according to De Meneghi et al. (2016), the negative impacts related to the possibility of contamination and public health are considered of high risk for treatments performed by dip-tank and portable manual sprayer. Therefore, this study aimed to evaluate the effect of the precipitation intensity and time on the efficacy of topical treatment of tick infestation.

Engorged females of *R. microplus* were obtained from different rural properties located in the central region of Rio Grande do Sul state, and the ticks had not been exposed to topical and injectable acaricides for at least 30 and 45 days, respectively. The engorged females were subjected to immersion tests following the method of Drummond et al. (1973), with modifications, when Reproductive efficiency (RE) and Reproduction inhibition percentage (INR%) were calculated:

RE = (weight of eggs × % of eggs for hatching × 20,000*) ÷ weight of engorged females

*20,000 larvae estimated in 1 g of eggs.

INR % = (RE of control group − RE of treated group)/RE of control group × 100.

First, the engorged females were washed in distilled water, dried with paper towels, and distributed in homogeneous groups with a maximum difference of 0.03 g between the groups.

Immersion tests were performed using Colosso (OuroFino Animal Health), a commercial product containing 30% chlorpyrifos, 15% cypermethrin, and 15% fenthion. Colosso FC30 was used according to the manufacturer's recommendations for immersion. Briefly, 25 μl of Colosso FC30 was diluted in 20 mL of distilled water. After solution homogenization, 10 female ticks from each group were immersed in the solution for 5 min. The negative control group (R1) consisted of ticks immersed in 20 mL of distilled water and ticks in the positive control group (R2) were immersed in Colosso FC30 solution only without subjecting them to rain bath.

The engorged females were divided into 18 groups (R1-R18). After immersion in Colosso, the tick groups were washed with distilled water for 30 min (R3 and R11), 1 h (R4 and R12), 2 h (R5 and R13), 3 h (R6 and R14), 6 h (R7 and R15), 9 h (R8 and R16), 12 h (R9 and R17), or 24 h (R10 and R18). In addition, precipitation at mild and intense was simulated for all the washing periods. To mimic intense precipitation, the females were immersed in distilled water for 5 minutes, while to mimic mild precipitation, a 15l backpack sprayer was used for 30 seconds.

After the simulation of precipitation, the engorged females were dried with paper towels, fixed in petri dishes with adhesive tape, and incubated at 28 °C and > 80 % humidity. The eggs from the engorged females with viable postures were weighed, and after 14 days of incubation, 0.3 g of the eggs were placed in glass tubes with hydrophobic cotton on the top. The glass tubes containing the eggs were incubated for 26 days, as described earlier, and hatchability analysis was performed. The efficacy of the chemical compound at different precipitation intensities and times was evaluated for each group.

Statistical analysis linear regression allowed us to evaluate the effects of rainfall intensity and exposure time on the product efficiency variable (INR). To adjust the regressions, dummy variables were created for rainfall intensity and non-significant categories at the 5% level were removed.

For product efficacy, the following final model was obtained $log(\text{INR}) = 4{,}59 - 0{,}0003x_1$, where x_1 indicates the exposure time. The diagnostic tests indicated that the model does not violate the normality assumption of the residuals (p-value=0.178) and that it is correctly specified (p-value=0.129). Furthermore, the fitted model explains R^2=42.23% of the *log*(INR) variability. Analyzing the values of the coefficients, it can be concluded that for each increase of one day in exposure to rain, there is a decrease of 0.03% in the efficacy of the product. None of the simulated precipitation intensity categories showed significant results. Thus, we can suggest that there was no dosage variation in the acaricide treatment (in the case of precipitation, underdosing) in addition to reducing possible environmental contamination resulting from precipitation.

The results obtained from the immersion test showed 100% efficacy for all the evaluated periods (30 min, 01, 02, 03, 06, 09, 12, and 24 h) and simulated precipitation intensity. Egg mass, fertility and reproductive index for each group are shown in Table 1.

The efficacy of acaricide treatment against ticks is directly linked to the mode of application, dispersion degree of active ingredients after application, and quality of chemical products (George et al., 2004). However, the role of precipitation in the efficacy of topical acaricide treatment remains unclear. Studies that have described the influence of precipitation on the efficacy of chemical products on cattle ticks are scarce, but some studies have shown that precipitation volume interferes with acaricide treatment (Davey et al., 2009; Zapa et al., 2020). However, the effects of precipitation in short time intervals after treatment have not been described.

Table 1

Evaluation of groups of Rhipicephalus microplus submitted to different simulated precipitation intensity and different exposure times to precipitation, based on reproductive efficiency and reproduction inhibition percentage

Identification group	Time	Precipitation intensity	Group weight (g)	Posture weight (g)	Hatching (%)	RE	INR%
R1		negative control	2.38	1.392	100%	11697.47	
R2		positive control	2.35	0.020	98%	166.80	98.57
R3	30 min	intense	2.36	0.027	99%	226.52	98.06
R4	1 hour	intense	2.38	0.025	98%	205.88	98.23
R5	2 hours	intense	2.35	0.028	99%	235.91	97.98
R6	3 hours	intense	2.37	0.027	99%	225.56	98.07
R7	6 hours	intense	2.36	0.030	99%	251.69	97.84
R8	9 hours	intense	2.40	0.027	99%	222.75	98.09
R9	12 hours	intense	2.39	0.028	99%	231.96	98.01
R10	24 hours	intense	2.37	0.040	99%	334.17	97.14
R11	30 min	Mild	2.40	0.025	99%	206.25	98.23
R12	1 hour	Mild	2.38	0.029	99%	241.26	97.93
R13	2 hours	Mild	2.41	0.031	99%	254.68	97.82
R14	3 hours	Mild	2.37	0.038	99%	317.46	97.28
R15	6 hours	Mild	2.36	0.029	99%	243.30	97.92
R16	9 hours	Mild	2.40	0.026	99%	214.50	98.16
R17	12 hours	Mild	2.41	0.033	99%	271.12	97.68
R18	24 hours	Mild	2.35	0.037	99%	311.74	97.33

RE= Reproductive efficiency, INR%= Reproduction inhibition percentage.

An *in vivo* study conducted by Davey et al. (2009) demonstrated that after animals were treated with coumaphos and submitted to different rain volumes, the chemical product showed weaker efficacy. However, in our study, the influence of precipitation from the difference in volumes was not observed.

Zapa et al. (2020) evaluated the influence of rain on fluazuron and fipronil treatments in cattle ticks. Precipitation intensity did not influence treatment efficacy in the different timelines (after 4h and 24h (Zapa et al., 2020). The results from the present study showed that simulated precipitation from 30 min after topical treatment did not interfere with treatment efficacy. Moreover, precipitation intensity did not influence treatment efficacy. In addition, subsequent precipitation simulations within 24 h after chemical treatment did not improve treatment efficacy.

The use and efficacy of Colosso® (OuroFino Animal Health) in cattle ranches in the southern region of Brazil have been widely discussed. A study that determined the efficacy of organophosphates and pyrethroids against *R. microplus* in Rio Grande do Sul state, Brazil, showed that despite the resistance of ticks to organophosphates, Colosso® (OuroFino Animal Health) demonstrated stronger efficacy (> 95 %) than other commercial formulations that did not contain fenthion (Reginato et al., 2017). However, the resistance of *R. microplus* from Rio Grande do Sul to cypermethrin, amitraz, chlorpyrifos, ivermectin, and fipronil was demonstrated by Klafke et al. (2017), and Ministério da Agricultura Pecuária e Abastecimento [MAPA] (1997) showed that the acaricide efficacy of these chemical compounds was lower than 95%.

According to FAO (2003), *in vitro* tests are important for monitoring the efficacy of acaricide chemical treatments. These tests facilitate appropriate product choice with the aim of reducing the number of treatments per year and reducing the selection pressure on resistant tick populations. Changes in the protocols of chemical treatment may reduce the efficacy of treatment, particularly protocol changes that involve the underdosing or overdosing of active ingredients.

Therefore, this study provides relevant information about the influence of precipitation on the efficacy of the topical treatment with Colosso FC30, which after simulated precipitations, did not show influence on posture when correlated to the control group with the one submitted to the drug, at different time intervals. Further studies on the influence of precipitation on acaricide efficacy should be carried out using other chemicals to develop excellent treatment protocols against *R. microplus,* given greater contributions to strategic tick infestation control.

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