ANTIPARASITIC EFFICACY OF FIPRONIL IN WISTAR RATS NATURALLY INFESTED BY *Polyplax spinulosa* (BURMEISTER, 1839)

EFICÁCIA ANTIPARASITÁRIA DO FIPRONIL EM RATOS WISTAR NATURAMENTE INFESTADOS POR *Polyplax spinulosa* (BURMEISTER, 1839)

ABSTRACT - Based on the importance of pediculosis by *Polyplax spinulosa* for rodents, this study evaluated two treatments against this louse in naturally infested Wistar rats. 21 adult male rats were randomly distributed into three groups (n = 7): T1 = treated with association of cypermethrin (5%), dichlorvos (45%) and piperidine butoxide (25%) administered topically by spraying; T2 = treated with fipronil 1 mg. Kg^{-1} administered topically by spot on; T3 = untreated animals (control). In the initial phase, animals were housed in cages and wrapped in white cotton fabrics for evaluating 4, 12, 24 and 48 hours post-treatment (HPT) to count the number of lice present in the external environment. In the second phase, the groups were transferred to polypropylene boxes for antiparasitic evaluation 7, 14 and 21 days post-treatment (DPT) by direct inspection of eggs and lice presence in the animals. The results showed higher lice counts 4 HPT, representing 41% of the total, and these findings were strongly influenced by the control animals with 20.51%. However, rats treated with fipronil had the highest counts with 24 HPT, representing 14.36% of lice. No variation in lice counts was observed in T1 (pharmacological association). The efficacy study in the animals revealed absence of lice and presence of eggs in treated animals (T1 and T2) at 7 and 14 DPT. In the 21st day, rats treated with the pharmacological association presented lice whereas fipronil treated rats remained only with eggs not hatched. However, this study demonstrated that Fipronil at 1 $mg.kg^{-1}$ via spot on is an effective option for the control of *P. spinulosa* in Wistar rats, while treatment with the association of cypermethrin (5%), dichlorvos (45%) and piperidine butoxide (25%) was not effective against eggs viability and hatching, resulting in the return of parasitic infestation.

KEYWORDS: phenylpyrazole**,** organophosphorus, pyrethroid, pediculosis

RESUMO- Baseado na importância da pediculose por *Polyplax spinulosa* em roedores, este estudo avaliou dois tratamentos para controle deste piolho em ratos Wistar naturalmente infestados. Para tal, 21 ratos adultos machos foram distribuídos em três grupos (n=7): T1= tratados com associação farmacológica cipermetrina (5%), diclorvos (45%) e butóxido de piperolina (25%), via tópica por pulverização; T2= tratados com fipronil 1 mg. Kg^{-1} via tópica por "Spot on"; T3= animais não tratados (controle). Na fase inicial do estudo, os animais foram acondicionados em gaiolas e envolvidos em tecidos de algodão branco para avaliação 4, 12, 24 e 48 horas pós-tratamento (HPT) do número de piolhos presentes no tecido (ambiente externo). Na

segunda etapa, os grupos foram transferidos para caixas de polipropileno para avaliação antiparasitária 7, 14 e 21 dias pós-tratamento (DPT) por inspeção direta da presença de ovos e piolhos nos animais. Os resultados revelaram maiores contagens de piolho 4 HPT, representando 41% do total, sendo estes achados fortemente influenciados pelos animais controles com 20,51%. Entretanto, ratos tratados com fipronil apresentaram as maiores contagens com 24 HPT representando 14, 36% dos piolhos. Não se observou variações nas contagens de piolho em animais do grupo T1 (associação de fármacos). O estudo de eficácia nos animais revelou ausência de piolhos e presença de ovos nos animais tratados (T1 e T2) no 7 e 14° DPT. Na avaliação realizada 21° DPT, ratos tratados com a associação farmacológica apresentaram piolhos enquanto ratos tratados com fipronil permaneceram somente com ovos não eclodidos. Contudo, este estudo demonstrou que o fipronil via "Spot on" em dose única na concentração de 1mg.kg^{-1} é uma opção eficaz para o controle de *P. spinulosa* em ratos Wistar, enquanto o tratamento com a associação de cipermetrina (5 %), diclorvos (45%) e butóxido de piperolina (25%) não se mostrou eficaz contra a viabilidade e eclosão dos ovos, resultando na reinfestação dos animais.

PALAVRAS-CHAVE: fenilpirazole, organosfosforados, piretróide, pediculose

INTRODUCTION

A survey conducted in the United States evaluated the prevalence of sanitary outbreaks in rodent facilities, revealing that infections of viral etiology decreased over the years, however, the casuistic of bacterial and ectoparasitic diseases remained constant, even with the use of sanitary barriers (MARX et al., 2017). According with these authors, the research credibility results depends on the control of external interferences which are not peculiarities of the experimental objective, considering that animals with infectious and/or parasitic diseases impair research findings, in addition to compromise the animal welfare.

Novergicus Wistar rat is one of the most breeding lines in rodent facilities, since they present desirable characteristics such as short breeding cycle, genetic similarity with humans, ease of maintenance and management (MATTARAIA et al., 2012). On the other hand, *Polyplax spinulosa* frequently parasite rats in rodent facilities, being an ectoparasite belonging to order Phthiraptera and suborder Anoplura (sucking lice), and the transmission between animals is by direct contact, causing irritation, restlessness and constant scratching, especially behind the ears.

Massive infestation can result in dermatitis and anemia (TAYLOR et al., 2017). In addition, *P. spinulosa* can act as a vector of *Mycoplasma haemomuris*, *Rickettsia typhi*, *Trypanosoma lewisi*, *Borrellia duttoni* and *Brucella brucei* (BAKER, 2006).

The use of antiparasitic drugs in sanitary management represents an important strategy in the control of ecto and endoparasites in veterinary medicine (CHARLIE-SILVA et al., 2018). Fipronil has been successfully used for the control of ectoparasites in cattle (SOUZA et al., 2014), dogs (DAVOUST et al., 2003; BONNEAU et al., 2010) and cats (PAYNE et al., 2001; SCARAMPELLA et al., 2005; KUŽNER et al., 2013), as well as the use of formulations composed by pyrethroids and organophosphates (SOARES et al., 2009, BELO et al., 2012, SOUZA et al., 2017).

Based on the importance of health management and the need to establish new therapeutic protocols for pediculosis in laboratory animals, this study aimed to evaluate the antiparasitic efficacy of fipronil 1% and the formulation composed by cypermethrin 5% + Diclorvos 45% + 25% piperonyl butoxide, both administered through topical route in Wistar rats naturally parasitized by *P. spinulosa*.

MATERIAL AND METHODS

For the study were used 21 male rats with 12 months of age (250g ± 50g), fed with Labina® commercial food and water *ad libitum*. Rats were reared under controlled temperature (24 to 28°C), relative humidity (50 to 70%) and photo period (12 light hours). The experiment was conducted at Brasil University, approved by the Institutional Ethics Committee for Research (process number 3042-3262 / 10), according to experimental protocols for good clinical practice (BRASIL, 2009).

Rats were naturally infested by *P. spinulosa* (Burmeister, 1839) (Figure 1) showing pruritus and bristling. The animals were randomly distributed into three groups of 7 animals each, constituting the treatments: T1 = cypermethrin + dichlorvos + piperoline butoxide; T2 = treatment with fipronil and T3 = untreated animals (control). The first therapeutic protocol consisted of administering the pharmacological combination: cypermethrin (5%), dichlorvos (45%) and piperidine butoxide (25%) prepared in the proportion of 1mL of the compound to 400 mL of water. The spray method was used to distribute the product over the entire body of the animal resulting in the concentration of 2.5 mg of cypermethrin, 22.5 mg of dichlorvos and 12.5 mg of piperidine butoxide per kilogram of body weight. The second treatment consisted of fipronil 1%, administered through topical route spot on, in the neck region at the concentration of 1 $mg.kg^{-1}$.

After treatment, each rat group was reared in an individual cage (60X40X30cm), wrapped in white cotton fabrics. At 4, 12, 24 and 48 hours post-treatment (HPT) the cotton fabric was removed from the cage, and immediately substituted with another. The removed tissues were evaluated by direct inspection and the respective parasite counts were determined. In order to evaluate the parasite presence in the animal body, the experimental groups were transferred to sterilized boxes and kept in different rooms with the same characteristics, to avoid cross infestation. After 7, 14 and 21 days post-treatment (DPT), each animal was observed by direct inspection to verify the presence of lice and eggs.

Lice counts were evaluated by a contingency table followed by a Chi-square Test, using the null hypothesis that the experimental groups did not differ, accepting a 5% of probability. Simple correspondence analysis was also performed to establish the inertia between the experimental data, in order to search for associative patterns between treatment and time analyzed for

parasitism. For evaluation over the days, descriptive statistics regarding the absence and presence of lice and eggs were also followed by multiple correspondence analyses.

The BoxCox test ($\lambda = 1$) and the Shapiro-Wilk test ($P > 0.05$) were used to establish the homoscedasticity and normality hypothesis of internally standardized residues. All statistical analyzes were processed in the SAS® software (Statistical Analysis System), version 9.3 (SAS, 2012).

RESULTS AND DISCUSSION

Contingency analysis (Table 1) correlates the number of lice found in the external environment with the expectation for that period (Chi-Square test = $p < 0.05$). The results revealed higher lice counts 4 HPT, representing 41% of the total. The control animals counts were the most significant, representing 20.51% and with a high number of lice (n = 40), when compared to the number of lice expected for this period (n = 32). However, 28 ectoparasites were counted for the rats treated with fipronil 24 HPT, ie, a higher expectation score (n = 19), even higher than the counts observed in the control group (n = 15) and treated with the pharmacological association (n = 12).

The correspondence analysis findings (Figure 2) corroborate the counts observed in the contingency study. It was also observed the correspondence of the control group 4 HPT, as well as of the group treated with fipronil 24 HPT.

The highest number of ectoparasites found in the control group 4 HPT was due to the fact that they had not received any treatment and the lice maintained with continuous blood spoliation. The continuous lesion stimulus suggests the hypothesis that the parasitism favored the

release of histamine, an important inflammatory mediator, which causes pruritus (HEYER et al., 1997), and associated to scratching there was a greater removal of lice to external environment.

Increase of lice 24 HPT observed in the treatment with fipronil is due to the drug characteristics which do not act by direct contact with the parasite body but by continuous intoxication during the parasitism. In addition, fipronil has been shown to play a role in the transport of chloride ions in the cell membrane through the γ-aminobutyric acid receptor (GABA) (ZHAO et al., 2003) and also acts on glutamate receptors coupled to chloride channels (NARAHASHI et al. 2010). The GABA neurotransmitter presence in the synaptic junctions results in uncontrolled central nervous system (CNS) activity and consequent hyperexcitability of the parasite (ZHAO et al., 2003). This effect on CNS may have contributed to the pronounced counts of lice in the external environment 24 HPT.

Differently to the results observed with the use of cypermethrin, dichlorvos and piperonyl butoxide treatment, which aims to act directly on the louse body, no significant variations was observed in the lice counts between the different periods. The organophosphorus dichloride bind the cholinesterase enzyme receptor, causing accumulation of acelticoline, resulting in motor incoordination and parasite death, while the pyrethroid type II cypermethrin inhibits the sodium channels, preventing the action potential and thus leading to a blockage of neural activity, also it can act on GABA type receptors only in high concentration (COSTA, 2008). Piperonyl butoxide presents synergistic effect on the cytochrome P450 system, decreasing drug metabolism by the liver. The joint action of these active principles did not result in significant changes in lice counts in the external environment.

All groups presented lower lice counts 48 HPT than the other observation periods (Table 1), probably due to lice removal in the counting procedure, with this management there was no possibility of lice returning to the host and the period was short for egg hatching. In the cycle of

lice, approximately 6 days are observed for egg hatching (TAYLOR et al., 2017) and the initial evaluation phase of this study contemplated only 2 days. On the other hand, the pruritus continued with lower intensity even after a decrease in the number of lice. This was due to the inflammatory response to repair skin lesions, possibly by continuous chemical mediators release from the inflammation (EMING et al., 2007), so dead or intoxicated lice continued to be taken in less quantity to the external environment in the act of scratching.

The parasitism comparative study (Table 2) revealed absence of lice and presence of eggs in all animals submitted to pharmacological treatment 7 and 14 DPT (Groups T1 and T2). In the 21st DPT evaluation, rats treated with the pharmacological association presented lice whereas firponil treated rodents remained only with eggs not hatched (Table 2).

Correspondence analysis evaluates the presence and absence of lice and/or eggs 7, 14 and 21 DPT (Figure 3), we observed a grouping between the observations of treatment with fipronil to the absence of lice and the possible presence of lice eggs, corroborating the findings described in Table 2.

The efficacy result was demonstrated by the individual evaluation over the days. Treatment with fipronil proved to be effective in the control of pediculosis by action on lice and egg of *P. spinulosa*. In the pharmacokinetic of fipronil, the drug accumulates in the cutaneous adipose tissue, favoring its excretion along with sebaceous secretion (COCHET et al., 1997), therefore, coming directly into contact with lice eggs and presenting a long residual period (FAO, 1998). Study conducted in rats with radiolabeled fipronil, administered by oral route at a dose of 4mg / kg, plasma peak was 5.5 hours after administration, elimination half-life of 183 hours in males and 245 hours in females, showing distribution throughout the body, but with a higher concentration in adipose tissue (FAO, 1998).

Pharmacological association treatment (T1) did not present an effect on eggs due to the appearance of new lice; this fact may be due to pharmacodynamic and pharmacokinetic characteristics, resulting in the low efficacy in the control of *P. spinulosa*, unlike the elimination characteristic of fipronil by sebaceous gland secretion (COCHET et al, 1997) that favored a long antiparasitic efficacy.

According to Marx et al. (2017), one of the most widely used active principles for treating lice in the United States is ivermectin, with a two-dose protocol ranging from 7 to 10 days. However, fipronil has been shown to be an excellent option as a single dose protocol for controlling *P. spinulosa* in Wistar rats. It is noteworthy that in agreement with the results of this study, after the 21 days of experimental design, all animals not treated with fipronil were submitted to the present treatment protocol, aiming to promote animal welfare in our laboratory.

CONCLUSION

This study demonstrated that fipronil at 1 $mg.kg^{-1}$ via spot on is an effective option for the control of *P. spinulosa* in Wistar rats, while treatment with the association of cypermethrin (5%), dichlorvos (45%) and piperidine butoxide (25%) was not effective against eggs viability and hatching, resulting in the return of parasitic infestation.

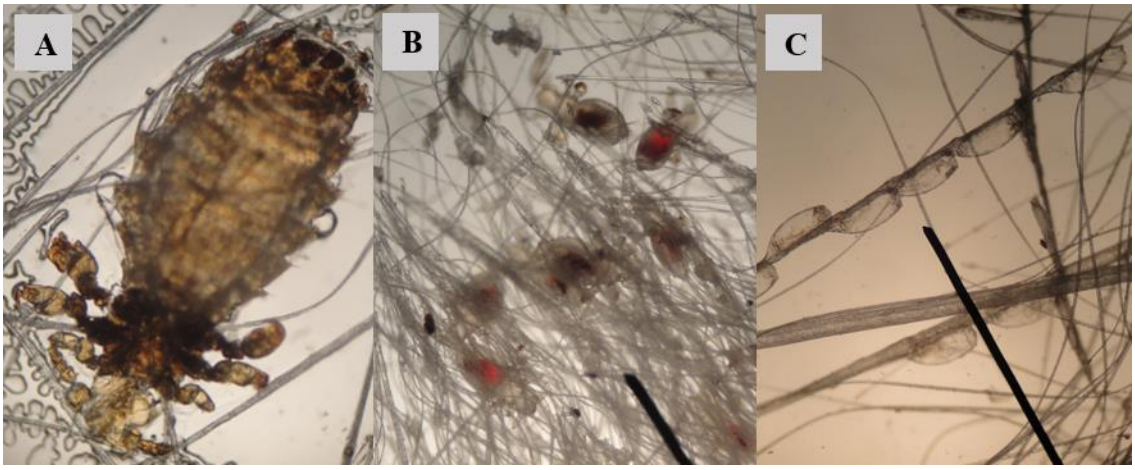

Figure 1. *P. spinulosa*: (A) Lice (100x magnification); (B) Lice (40x); (C) Eggs (100x).

Table 1: Frequency and expectation of lice count from the external environment of wistar rats submitted to several treatments

Treatment	Hours				
Frequency **Expected** **Percent**	**4**	**12**	**24**	**48**	**Total**
Control	40 32.82 20.51%	16 14.36 8.21%	15 22.56 7.69%	9 10.26 4.62%	80 41.03%
Pharmacological association*	22 19.28 11.28%	7 8.43 3.59%	12 13,25 6.15%	6 6.02 3.08%	47 24.10%
Fipronil	18 27.89 9.23%	12 12.20 6.15%	28 19.18 14.36%	10 8.71 5.13%	68 34.87%
Total	80 41.03%	35 17.95%	55 28.21%	25 12.82%	195 100.00%
Probability Chi-Square: 0,0438					
***Cypermethrin, dichlorvos and piperonyl butoxide**					

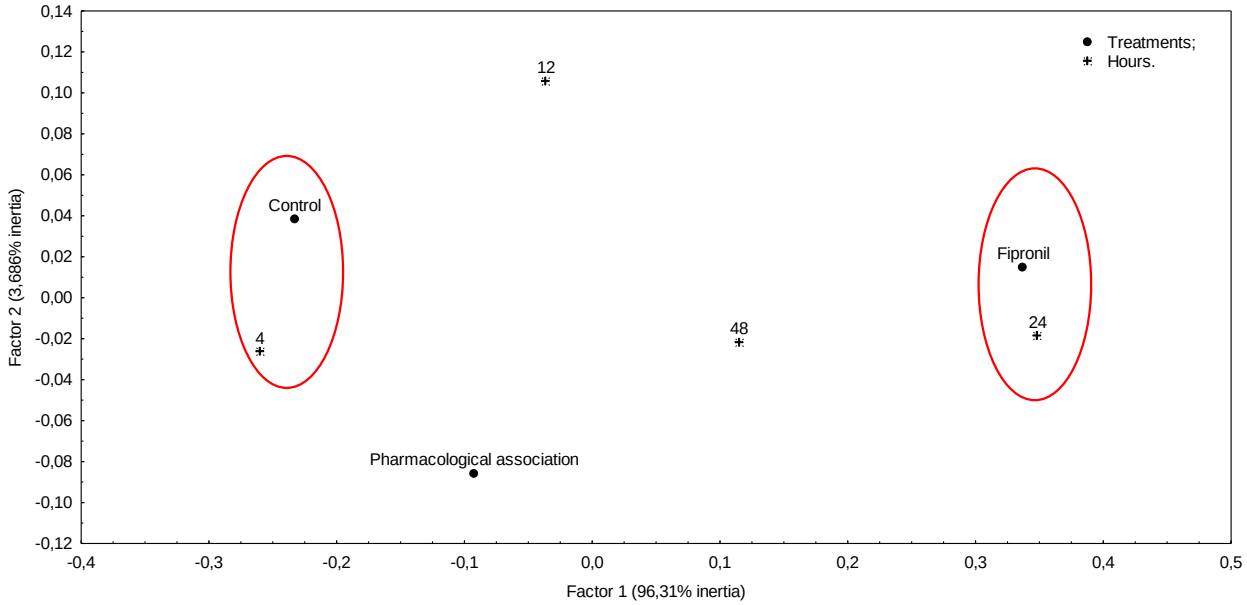

Figure 2: Behavior patterns between the treatments and the periods analyzed for control of *P.spinulosa* in wistar rats by correspondence analysis.

Table 2: Presence or absence of adult lice or eggs by individual direct examination in wistar rats submitted to several treatments.

Treatment	**Days**		
Lice **Eggs**	**7**	**14**	**21**
Control	present	present	present
	present	present	present
Pharmacological association	absence	absence	present
	present	present	present
Fipronil	absence	absence	absence
	present	present	present

***** Cypermethrin, dichlorvos and piperonyl butoxide

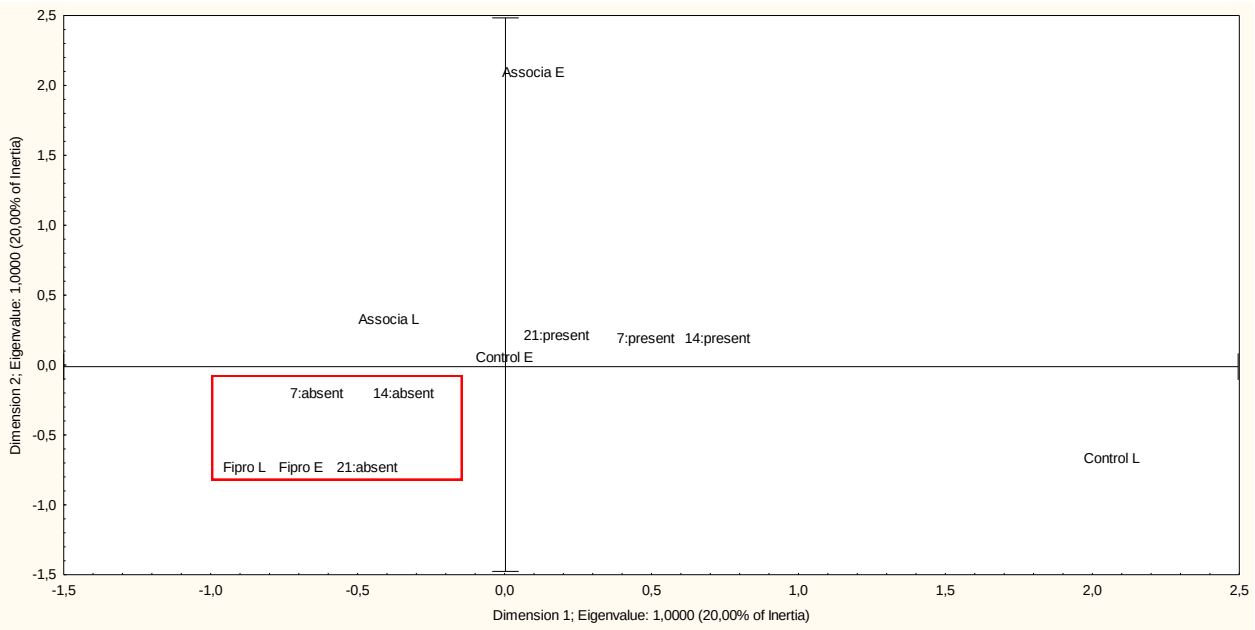

Figure 3: Patterns between treatments: pharmacological association (Associa), Fipronil (Fipro) and control (Control) for presence or absence of lice (L) and eggs (E) over the days analyzed: 7, 14 and 21 days in wistar rats naturally parasitized by *P.spinulosa* in correspondence analysis.