

Efficacy of 1% Permethrin and 2% Permethrin Combined with 1% Piperonyl Butoxide against *Rhipicephalus sanguineus* Ticks in Naturally Infested Dogs

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Abstract

The aim of the present study was to compare the ixodicide efficacy of 1% permethrin applied as a shampoo-bath in single treatment and a combined formulation of 2% permethrin + 1% piperonyl butoxide applied as powder in a single treatment against *Rhipicephalus sanguineus* ticks, in naturally infested dogs. Eighteen adult cross-mixed dogs, harboring to at least 20 ticks/animal, were selected on pre-treatment live tick counts. On day 0, the dogs were then divided in 3 groups of 6 animals each, for treatment: Group 1 (G1) received 1% permethrin applied as shampoo-bath (approximately 10 ml/kg bw), according to label instructions. G2 was treated with 2% permethrin + 1% piperonyl butoxide applied as powder, and rubbed as massage on the body, as recommended by the producer. G3 served as a non-treated control. After the treatment, the dogs were checked out for live ticks counting on days 0, 1, 7, and 14. The ixodicide efficacy was assessed based on the percentage reductions of ticks on the days analyzed post-treatment, with respect to the untreated control. Results showed that permethrin alone removed ticks on 81%, 93.1% and 89.6% for days 1, 7 and 14, respectively. The combined formulation of 2% permethrin + 1% piperonyl butoxide exerted a better efficacy of 98.8, 97.7 and 97.0%, respectively. It is concluded that this combined formulation was highly effective at rapidly repelling and killing *Rhipicephalus sanguineus* ticks on naturally infected dogs, showing a significant effect on days 1, 7 and 14 after tick exposure. The sustained high and quick level of efficacy of this combination may well interfere with the transmission of *Rhipi-*

cephalus sanguineus ticks. It is concluded that the sustained high level of efficacy of the permethrin + piperonyl butoxide combination may well exert an additive effect and could interfere with the transmission of *Rhipicephalus sanguineus* ticks.

Keywords

Efficacy, Permethrin, Permethrin + Piperonyl Butoxide, *Rhipicephalus sanguineus*, Dogs

1. Introduction

Ticks are obligate hematophagous ectoparasites [1] and are the most important vectors of pathogens within the phylum Arthropoda [2]. Worldwide, there are 84 species [3], with *Rhipicephalus sanguineus*, also known as the brown dog tick, being the one with the greatest distribution [2]. In Mexico, *R. sanguineus* is one of the most important species and has been identified in Cuernavaca, Morelos, (central part of Mexico), Mexicali, Baja California and Culiacán, Sinaloa (north-west part of Mexico), among others [1]. In addition, *Rhipicephalus sanguineus* has been often found in other hosts, including humans [1] [2]. It is a vector of different pathogens, such as *Ehrlichia canis*, *E. chaffeensis*, *Babesia canis*, *B. vogeli*, *Rickettsia rickettsii*, *R. conorii*, *Coxiella burnetti* and *Hepatozoon canis*, some of them of zoonotic importance [1]-[3].

The control of ticks is based mainly on chemical treatment, through synthetic acaricides applied by spraying, in baths or spray, and in subcutaneous injections.

Some of these chemicals also have a persistent effect lasting for several weeks after their initial application [4], while others are effective for several months [5].

On the other hand, permethrin is an insecticide belonging to the group of pyrethroids, which act at the level of the insect's nervous system, producing a series of disordered nervous impulses, accelerating tissue degeneration, and inhibiting their feeding process [6]. It is of low toxicity for mammals since it is excreted quickly after oral or topical exposure [7].

Various studies have verified the effectiveness of permethrin against the brown dog tick in different presentations and concentrations, reaching from 60.8% to 100% efficacy, as a single treatment or in combination with other acaricides [3] [4] [8] [9].

Another control strategy has been the use of ixodicides with synergists, which are not considered toxic by themselves [10]; intervene on different enzymes in order to potentiate the effect of ixodicides, such as piperonyl butoxide [10] [11], which acts at the microsomal level as inhibitor of cytochrome P450 monooxygenases [11], causing the inhibition of the oxidative functions present in the metabolism of pyrethroids [12] [13].

The aim of the present study was to compare the efficacy of a single treatment of 1% permethrin applied as a shampoo-bath, versus a 2% permethrin combined

formulation of 1% piperonyl butoxide applied as powder, against *Rhipicephalus sanguineus* ticks in naturally infested dogs.

2. Materials and Methods

2.1. Study Location

The study was carried out at the “Huellitas” dog shelter, located in the State of Morelos (central Mexico).

2.2. Animals

Eighteen adult mixed breed dogs, harboring to at least 20 ticks/animal, were selected on pre-treatment live tick counts. The dogs were chosen regardless of sex, breed, or age. They were housed in booths of approx. $90 \times 90 \times 120$ cm and each group was in 3 pens of approximately 6×8 meters each, ensuring that there was a distance of at least 10 meters between each pen. Animals were fed on Royal Canin food and water was provided *ad libitum*.

2.3. Drugs

1% permethrin for a shampoo-bath single treatment.

2% permethrin combined with 1% piperonyl butoxide for single powder treatment. Both formulations were provided by Laboratorios Salud Animal, S.A de C.V.

2.4. Experimental Design

On day 0, the dogs were then divided in 3 groups of 6 animals each, for treatment:

Group 1 received a single treatment with 1% permethrin applied as shampoo-bath (approximately 10 ml/kg bw), according to the producer's instructions.

Group 2 received a single treatment with 2% permethrin combined with 1% piperonyl butoxide applied as powder, and rubbed as massage on the body, as recommended by the producer.

Group 3 served as a non-treated control.

2.5. Dogs Monitoring.

After the treatment, the dogs were checked out for live ticks counting on days 0, 1, 7, and 14.

2.6. Efficacy

The ixodicide efficacy was assessed based on the percentage reductions of ticks on the day's analyzed post-treatment, with respect to the number of live ticks present on the untreated control. It was calculated using the following formula [14]:

Efficacy = Arithmetic mean number of live ticks (Control) – Arithmetic mean number of live ticks (Treated) $\times 100$.

Arithmetic mean number of live ticks (Control).

2.7. Statistical Analysis

Comparison of the efficacies of the treatments with the untreated control groups was carried out, using the Kruskal-Wallis test [15].

3. Results and Discussion

A summary of the tick counts and efficacy results are shown in **Table 1** and **Table 2**.

Table 1. Number of ticks observed in the experimental dogs before and after treatment with permethrin alone or a combined formulation of permethrin + piperonyl butoxide.

Group no. (6 dogs/group)	Sex	Weight (kg)	Route of administration (mg/kg/bw)	Number of ticks on day			
				0*	1	7	14
1 1% permethrin	H	25	Shampoo bath 10 ml/kg/bw	100	27	6	11
	M	17		50	42	20	13
	M	22		40	10	6	19
	M	20		30	7	1	5
	H	14		75	10	3	3
	M	23		25	7	1	5
Total				320	103	37	56
2 2% permethrin+ 1% piperonyl butoxide	M	25	Powder sprinkled <i>ad libitum</i>	40	2	0	0
	H	18		27	4	2	2
	M	28		22	0	2	7
	H	25		25	0	0	1
	H	30		30	0	1	3
	M	13		20	0	7	3
Total				164	6	12	16
3 Untreated control	H	30	-----	250	70	59	98
	H	20		200	17	25	11
	M	20		20	7	9	5
	H	17		20	16	8	11
	M	18		28	35	29	14
	H	20		25	9	9	8
Total				543	154	139	147

Note: *Day of treatment.

Table 2. Efficacy of 1% permethrin applied alone as shampoo or a combined formulation of 2% permethrin+ 1% piperonyl butoxide applied as powder in dogs naturally infected with *Rhipicephalus sanguineus* ticks.

Group no.	Compounds	Efficacy on days			
		0	1	7	14
1	1% permethrin	0	81a	93.1	89.6 ^a
2	2% permethrin + 1% piperonyl butoxide	0	98.8	97.7	97 ^b
3	Untreated control	-----	-----	-----	----- ^a

Note: ^{a,b}Different letters in the same column represent statistically significant differences ($p < 0.05$) between average effectiveness.

Group 1 (permethrin applied as shampoo-bath) showed a remarkable reduction of ticks, observing that of 320 specimens on the group counted at day 0, this number decreased to 103 on day 1 and to 37 ticks on day 7 after treatment. By day 14 after initial of the study, the number of ticks observed increased to 57, indicating that the initial effect of the compound has worn of **Table 1**.

In terms of efficacy, the percentage obtained for this group was 81.0% for day 1 and 93.1% for day 7. For day 14 after treatment, the efficacy conferred was reduced to 89.6%, suggesting that tick-killing activity of shampoo had decreased (**Table 2**).

Group 2 (2% permethrin + 1% piperonyl butoxide applied as powder) showed a drastic decrease in ticks, where of 164 specimens observed at day 0, this count was reduced to only 6 ticks. On day 7 after treatment, the specimens observed were 12. On day 14 after treatment, the number of ticks increased to 16, indicating that in the same way, the effect of the combined application of permethrin + piperonyl butoxide was already declining (**Table 1**).

The efficacy conferred for this group was 98.8%, 97.7% and 97.0%, for days 1, 7 and 14, respectively (**Table 2**).

In relation to Group 3 (non-treated control), the tick count was always high, recording a total number of 543, 154, 139 and 147, for days 0, 1, 7 and 14 after treatment, respectively. Here, the tick counts recorded throughout the study on the untreated dogs, confirms that the study complies with the guideline recommendations suggested by [14].

Forty-five days after the treatment, all dogs were showing increasing numbers of ticks, since the study area is highly prevalent in these arthropods.

It is important to note that no adverse health effects related to the treatment occurred during the study.

The statistical analysis indicated that significant differences were found between the experimental groups (G1 - G2) in the count of ticks retrieved for days 1 and 14, but not for day 7 ($p < 0.05$); regarding control group (G3), significant differences were found between it and G2 for days 1, 7 and 14, and between G3 and G1 only on day 7 ($p < 0.05$).

Several chemicals, or combinations of chemicals, with acaricidal or insecticidal properties and which are appropriate and safe for treatment of domestic dogs and cats, have been formulated for application either orally, parentally, topically, or as medicated collars [16] [17].

Permethrin has been a widely used acaricide for control and prevention of parasites in both dogs and large animals [6] [10] [18]. In dogs, it is most administered as topical treatment via spot-on, although it has also been formulated as collars. The active ingredient is distributed through the coat from the application site and along stratum corneum, however, studies suggest that the most distal parts may not receive the same concentration of the product as those closest application site [19].

In the present study, two products applied topically were compared to review the tick-killing efficacy of permethrin alone at 1%, applied as shampoo-bath and the combination of 2% permethrin with 1% piperonyl butoxide powder. In the case of these two presentations, they allow a more uniform distribution since they are applied throughout the animal's body and not in a single place, which could ensure adequate drug concentrations in the sites where ticks commonly lodge, such as interdigital spaces, armpits, groin and ears.

The results obtained demonstrated a greater efficacy with the combination of permethrin and piperonyl butoxide at day 1 (98.8%), although for days 7 and 14, the effect was in slight decline (97.7% and 97%, respectively), the efficacy remained above 90%, unlike 1% permethrin applied alone, which on day 7 obtained an efficacy of 99.8%, however by day 14 it decreased to 89.66%.

These percentages coincide with diverse studies in which the effectiveness of permethrin in different combinations has been analyzed, with the efficacy and residual effect of imidacloprid + permethrin being one of the most studied to measure. Epe *et al.* (2003) [20], found that this combination offered a preventive efficacy of 94% at day 9 post-application as spot-on and remained the efficacy above 90% against *R. sanguineus* for a period of five weeks. Otranto *et al.* (2005) [21], also recorded that this combination got an efficacy above 95% against ticks on naturally infested dogs from day 14 to final day of observation period (56 days). In another study of [22], compared three commercial products applied as spot-on, two of them including permethrin as an active ingredient (imidacloprid/permethrin, IP and dinotefuran/pyriproxyfen/permethrin, DPP) which achieved an efficacy of 99.6% on day 9 post-infestation, reducing this percentage till day 29 to 96.7% for the DPP treated group and to 91.7% for the IP treated group.

Following the same line, the combination dinotefuran/pyriproxyfen/permethrin was challenged by [23], where the immediate efficacy was 79.9% and the persistence of efficacy above 90% sustained for three weeks.

Other combinations have been examined, such as methoprene/permethrin, which achieved an efficacy greater than 90% at four weeks and with a residual effect that remained for 28 days [8], and fipronil/permethrin [24], where the acaricidal effect started at 4 h after treatment with a percentage of $\pm 94.7\%$ from day 2 to day 21 postexposure.

On the other hand, the results of the present study suggest the synergistic action of piperonyl butoxide, coinciding with previous studies [25], where it was demonstrated that the combination of amitraz and piperonyl butoxide acts additively against *Boophilus microplus* ticks. In another more recent study [26], it was shown that the enzymatic activity of oxidases as a mechanism of resistance to ixodicides when verifying the synergistic effect of piperonyl butoxide with cypermethrin, generating the inhibition of this activity in *R. sanguineus* larvae.

It is worth highlighting the importance of maintaining high percentages of efficacy for a long period; however, it is also necessary to consider a short latency period, to reduce the risk of disease transmission by vectors. Horak *et al.* (2012) [23], mentioned that tick burdens exceeding 10 individuals after treatment are quite adequate for the transmission or acquisition of organisms responsible for tick-borne diseases in the field.

A reduction in parasite numbers will inevitably influence the prevalence of the diseases that they transmit. It is also possible that chemicals that have a repellent, or a particularly rapid killing effect, could eliminate ticks before they can transmit vector-borne organisms with which they may be infected. For instance, *R. sanguineus* is a vector of *Babesia canis*, *Babesia vogeli*, *Babesia gibsoni*, *Hepatozoon canis* and *Ehrlichia canis*, the causative organisms of tick-borne diseases that affect dogs in different regions of the world [27].

Permethrin has been compared with other ixodicides such as fipronil, fluralaner, afoxolaner or sarolaner to determine the time of onset of action; here [28] found this beginning of activity two hours after spot-on application with an effectiveness of 36.9%, which slowly increased in the following 48 hours to 80.1%, in this study, permethrin in combination with imidacloprid failed to reach the minimum percentage to be considered the first option, since it is also mentioned, in general terms, that the time necessary for the transmission of pathogens through an infected tick can be 24 - 48 hours after feeding. The same product was analyzed by [29], in a study to verify its effectiveness against the transmission of *Ehrlichia canis* by *R. sanguineus* in dogs, resulting in 100% blocking of transmission.

Although the latency period or effectiveness against disease transmission was not analyzed in the present study, the results obtained on day 1 post-treatment in Group 2 suggest a high probability of disease prevention, since a total of 6 live ticks were counted (98.8% of efficacy), which means a lower risk of infection.

Several chemicals, or combinations of chemicals, have been formulated for application either orally, parentally, topically, or as medicated collars.

Since pet owners do not comply with the recommended time periods between the administrations of these tick remedies, serious gaps in efficacy may occur. However, in some populations, the indiscriminate use of this type of control has generated resistance to certain active ingredients, as well as it can cause a negative environmental impact, potentially affecting the natural insect fauna [26] [30].

Besides, it is expected that dogs are inevitably going to be washed or sham-

pooed, or swim or go out into the rain while being walked by their owners or working in the field. Therefore, the activity of any treatment applied may lead to a reduction of activity of the drug used.

Under the conditions in which the present study was carried out, it was observed that the combination of 2% permethrin + 1% piperonyl butoxide produces additional efficacy that benefits the treatment of *R. sanguineus* infections in naturally infested dogs.

It is worth highlighting the importance of always maintaining assertive and effective communication between veterinarians and pet guardians, as well as considering the rational use of chemical products to ensure the correct use of the ectoparasiticide, reducing the gaps in efficacy and the negative effects that could be caused by mishandling.

4. Conclusions

A combination of 2% permethrin + 1% piperonyl butoxide applied as a single powder treatment was highly effective at rapidly repelling and killing *Rhipicephalus sanguineus* ticks on naturally infected dogs, showing a significant effect on days 1, 7 and 14 after tick exposure.

The sustained high and quick level of efficacy of this combination may well interfere with the transmission of *Rhipicephalus sanguineus* ticks.

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Availability of Data and Material

All datasets are included in this manuscript.

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Authors' Contributions

AMA contributed to the study design, carried out the field study, ticks counting and results interpretation. FIV contributed to the study design, carried out the field study, ticks counting, results interpretation, manuscript revision and discussion. JAM collaborated with the field study, ticks counting and results interpretation and provided financial support. RA-G carried out the statistical analysis. All authors read and approved the final version of the manuscript.

Animal Research

The work was carried out adhering to the guidelines of the Institutional Committee for Use and Care of Experimental Animals of the institution, according to the Mexican Official Regulation NOM-062-ZOO-1999 and Animal Research: Report-

ing of *in Vivo* Experiments guidelines followed.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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