Efficacy and Safety of Topical Fipronil 1% and Deltamethrin 0.1% in Water Buffaloes (*Bubalus bubalis*) Naturally Infested by *Haematopinus tuberculatus*

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Abstract: *Haematopinus tuberculatus* is the main arthropod affecting buffaloes around the world. It causes intense itching and restlessness, altering feeding habits, thus reducing animal productivity. This parasitosis is seldom studied, as it does not lead to significant animal mortality or financial losses. In this project, we evaluated the efficacy and safety of two commercially available products for control of pediculosis in naturally infested buffaloes. A total of 24 milk producing, clinically healthy female buffaloes that were naturally infested by lice were included in this study. Animals were equally distributed into two groups: group 1 was treated with a single dose of *pour on* Fipronil (1%, Carval de Colombia, 1 ml of product per 10 kg of live weight). Group 2 was treated with a single dose of deltamethrin (5%, Bioara, SA), at a final concentration of 0,1% applied with an aspersion pump as a single bath of 4 liters of diluted solution per animal. Lice were counted weekly from day 1 to day 58 post-treatment. Up to day 23 post-treatment, no significant differences in efficacy were observed between products.Fipronil 1% consistently exhibited an efficacy above 70% from day 8 and until 30 day of the study. These results suggest that both products are efficient and safe to control lice infestations in buffaloes. However, fipronil 1% exhibited a greater residual effect, which reduces the number of baths required to treat infested animals, and reduces the probability of selecting for drug-resistant lice populations.

Keywords: Ectoparasites, pediculosis, buffaloes, treatment, effectiveness.

INTRODUCTION

Lice are one of the main ectoparasites affecting water buffaloes in many countries. Typically, infestations are caused by *Haematopinus tuberculatus* [1-12], an insect belonging to the Anoplura suborder. These lice have sucking-type mouthpieces, flat body, and adults can reach sizes of up to 3.5 mm, which makes them easy to diagnose since they are visible to the naked eye [13]. *Haematopinus tuberculatus* is a permanent parasite with a simple life cycle that is completed in their host between days 21 and 27, and consists of the egg stage, three nymphal instars, and finally the adult louse. They can only survive for a short period of time in the environment [2, 12, 14].

Presence of lice on the animal's body causes skin irritation, intense itching, and blood loss due to haematophagous activity of *H. tuberculatus*, which may ultimately lead to anemia. In addition, the main cause of loss of animal productivity is the intense itching since it reduces its time for grazing, and may cause lesions that can be subsequently be infected by *Cochlyomyia hominivorax* and *macellaria*, which cause myiasis and worms [13, 15]. Furthermore, studies have suggested that these insects can be vectors for pathogens such as *Brucella abortus* [14] and *Anaplasma marginale* [16], highlighting the need to control this parasite.

Among the alternatives used to control these arthropods are natural products such as *Artemisia* extract, *Lupinus* seed powder [17], and esential oils [18]. Traditional pharmaceutical approaches have implemented the use of macrocyclic lactones such as ivermectin [19, 20], eprinomectin [5], deltamethrin [1], albendazole, coumaphos [21], and other chemical products such as malathion and diazinon [22], with the aggravating factor that most of them generate high levels of contamination in foods of animal origin.

To date, there are few available registries on the efficacy and safety of ectoparasiticides used in water buffaloes. Therefore, in this study we have compared the pediculicidal efficacy and dermal safety, under field conditions, of 1% fipronil 1%and deltamethrin 0,1% in controlling lice naturally infesting water buffaloes (*Bubalus bubalis*).

MATERIALS AND METHODS

The institutional Animal Care and Use Committee (CICUA) at CES University (Antioquia, Colombia) approved this study, as registered in committee minute

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number 11, 2015. This study was performed between March and June 2015 in two commercial water buffalo farms located at 08º 34' N, 75º 31' W, and at 50 meters above sea level and with an average temperature of 27 ºC.

A total of 24 female milk-producing Murrah buffaloes at the same lactation stage, with an average age of 5,5 years, and average weight of 520 kilograms, were used in this study. All animals presented natural infestation by lice and were free from other diseases. Participating animals were kept under the same conditions as the rest of the population, during grazing and had *ad libitum* water intake. In addition, because these buffaloes were lactating, their feed was supplemented with pellets during milking, and they were milked twice a day.

Determination of Infestation and Sampling for Taxonomic Identification

On day 0, each animal was specifically examined in eight sites reported as predilection sites for lice infestation by the World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P) [23]. The examined sites are shown in Figure **1**.

For the taxonomic identification of the collected specimens, previously described taxonomic keys [12, 24, 25] were used and compared to morphologic descriptions published by Manning and Graham, 1849 [26].

Study Groups

Participating animals were distributed in two groups, one for each treatment. One group (**Group 1**), consisting of 12 female buffaloes, was treated following manufacturer's instructions for bovine; a single dose of Fipronil (1%, Carval de Colombia) of 1 ml of product per 10 kg of live weight, poured on the animal's back – the space between the top of the shoulders and the tail head. **Group 2**, also consisting of 12 female buffaloes, was treated with aspersion baths of deltamethrin (5%, Bioara, SA), at a final concentration of 0,1% applied with and aspersion pump as a single bath of 4 liters per animal.

Post-Treatment Lice Count to Assess Product Efficacy and Side Effects on Skin

In order to assess the efficacy of the tested products, the same team member by using a 10X magnifying glass and a powerful light source, counted lice at the eight sites of predilection on days 8, 15, 23, 30, 37, 44, 51, and 58 post-treatment. To quantify the efficacy of each product, the Abbott formula [27] was adapted to evaluate the percent reduction of lice as follows:

$$Efficacy(percentreduction) = 100 \times \frac{(C - T)}{C}$$

In which C is the lice count at day 0, before treatment; and T is the lice count in treated animals on the specified day post-treatment.

On the first day of treatment, following the methodology proposed by Draize, 1944 [28], each animal was evaluated at 2 and 4 hours after insecticide administration in order to search for skin reactions. This was continued during the length of the study, when

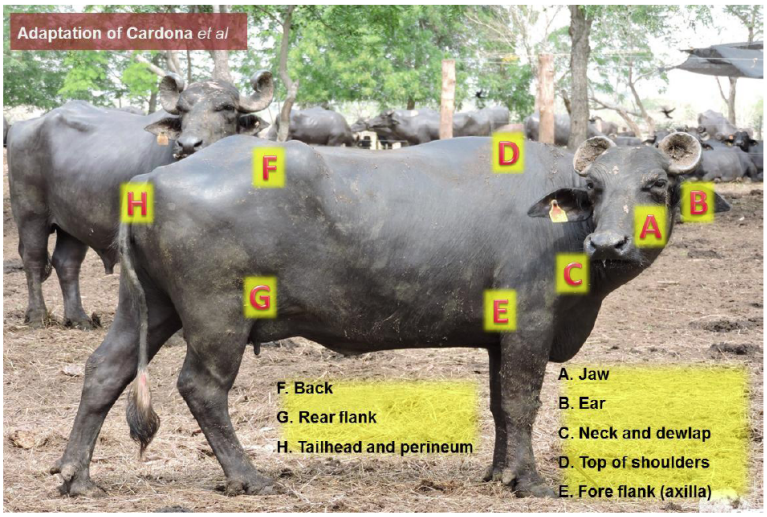

Figure 1: Predilection sites for lice infestation by the World Association for the Advancement of Veterinary Parasitology **(WAAVP)** (Holdsworth *et al*., 2006) [23].

animals were being evaluated once a week for lice infestation levels and product efficacy.

Statistical analysis was performed using Statgraphics Centurion XVI software, and groups were compared by paired t-test.

RESULTS

Infestation Levels, Taxonomic Identification, and Product Safety

All collected lice were taxonomically classified as *Haematopinus (H) tuberculatos*. All participating animals exhibited severe levels of infestation by these lice. However, the distal portion of the tail was consistently the site where parasite number was highest. Regarding product safety, both products were well tolerated by animals, as no adverse skin reaction was attributed to the use of these insecticides.

Product Effectiveness

The site with the highest parasite prevalence was the distal portion of the tail (45%), followed by the tail head and perineum (11%), jaw (10,4%), and ears (8.1%). The efficacy of single treatments of lice with fipronil and deltamethrin are shown in Table **1**.

Table 1: Percent Lice Reduction (Mean ± S.D.) in Female Lactating Water Buffaloes Treated with Fipronil 1% or Deltamethrin 0.1% for 58 Days under Field Conditions

Day	Group 1 (Fipronil 1%)	Group 2 (Deltamethrin 0.1%)
8	96,35% ± 9%	87,55% ± 32%
15	87,47% ± 15%	59,42% ± 62%
23	73,10% ± 48%	1,02% ± 127%
30	88,70% ± 15% *	2,10% ± 87% *
37	26,87% ± 88%	- 80,08% ± 223%
44	- 82,09% ± 418%	- 39,20% ± 174%
51	- 164,8% ± 491%	- 24,43% ± 373%
58	- 35,85% ± 197%	- 20,30% ± 538%

*Indicates statistically significant difference (p≤0,05). (Negative results indicate infestations greater than those of day 0 (study start)).

As shown in Table **1**, both treatments reduced lice infestation in treated animals, and while on day 23 the reduction percentages were greater in the group treated with fipronil 1% than in those treated with deltamethrin 0.1%, this was not statistically significant (p>0,05). On day 30, the group treated with fipronil 1% exhibited a reduction of 89%, while the group treated with deltamethrin 0.1% exhibited a 2% reduction in lice infestation, being this difference between groups statistically significant (p≤0,05).

After day 30, percent infestation reduction values rise to levels higher than those observed at day 0. There was no statistically significant difference between groups at this time point (p>0,05), or the remainder of the study.

Regarding skin reactions, we did not observe skin reactions that were attributable to the use of these insecticides in studied animals.

DISCUSSION

Similar to other bovines, water buffaloes have gregarious habits, which in addition to the form of exploitation –mainly extensive- and the large number of animals, favors the transmission of parasites such as lice. Consistent with our findings, *H. tuberculatus* has been previously reported as the most prevalent species in these hosts in studies performed by different research teams at different geographic locations such as those by Mamun *et al*., 2010; Veneziano *et al*., 2007, 2004, 2003; Munoz *et al*., 1987; Meleney and Kim, 1974; Piotrowski, 1974; Chaudhuri and Kumar, 1961, and more recently by Figueiredo *et al*., 2013; Kumar *et al*., 2015 [1-6, 9-12]. Nonetheless, additional studies have reported *Haematopinus quadripertusis* and *Haematopinus eurysternus* as the most prevalent species in buffaloes in some Asian countries.

In this study, both *Pour On* fipronil 1% and deltamethrin 0.1% aspersion bath showed an efficacy peak at day 8 post-treatment, leading to 96,3% and 87,5% reduction of lice infestation, respectively. Similar results regarding the rate of lice infestation reduction were reported by Tarek *et al.,* (2013) [29]. In that study, efficacy of malathion 0,057%, diazinon 0,015%, and diazinon 0,06% EC, was assessed in 20 buffaloes, and for all treatments the peak efficacy was observed between days 5 and 7. However, compared to our results, the achieved infestation reduction percentages in that study were lower than those reached in our study, being 55,4% for malathion 0,057%; 28.7% for diazinon 0,015%; and 63,8% for diazinon 0,06% EC.

Studies on the use of pyrethroids, such as deltamethrin, for controlling *H. tuberculatus* infestation in buffaloes are scarce. In our study, animals treated with a working dilution of deltamethrin at 0,1% exhibited gradual reinfestation after day 30 post-

treatment, reaching high numbers of parasites at the end of the study (day 58). On the contrary, [30], reported an efficacy of 100% from day 7 to 63 post-treatment for prolonged action alpha-cypermethrin at 1,5%, applied at 0,02 mg/buffalo for control of *Haematopinus tuberculatus*. In addition, in that study productivity and reproductive parameters were also assessed.

Similarly, Veneziano *et al*., 2013 [31], assessed the efficacy and safety of *pour-on* alpha-cypermethrin for the treatment of *Haematopinus tuberculatus* in 20 buffaloes with an average age of 7,2 years. The authors reported this product as completely effective (100%) at day 7, highly effective (99,8%) at day 14, and completely effective (100%) at day 21 post-treatment, and up to the end of the study (day 56). Using topical administration of cypermethrin 0,5% for control of *Haematopinus tuberculatus* in buffaloes, [32], reported an efficacy of 94,7% at day 28 post-treatment, which differs from our results since reinfestations were noticeable from day 30 post-treatment up to the end of the study (day 58.

Fipronil is a member of the phenylpyrazole chemical family. Phenylpyrazoles share mechanism of action with macrocyclic lactones, since fipronil binds to internal chloride receptors, inhibiting cellular ion flux and abolishing neuroregulatory function of gamma-aminobutyric acid (GABA), thus leading to hyper-excitation, spastic paralysis, and subsequent parasite death [33, 34]. Previous studies have evaluated the use of macrocyclic lactones for controlling *Haematopinus tuberculatus* in buffaloes, such as that by Didonet and Nagendra, 1985, in which the efficacy of two doses of subcutaneously injected ivermectin were tested; 0,2 mg per kg live weight and 0,4 mg per kg live weight. The higher dose, 0,4mg/kg, showed and efficacy of 100, 100, 70, 50, and 50% at days 7, 14, 21, 33 and 45 post-treatment, respectively. Similarly, Lau and Singh, 1985, assessed the efficacy of subcutaneously injected ivermectin at 0.2 and 0.4 mg/Kg body weight, and observed lice reduction rates of 85% and 100% at day 7 post-treatment, respectively. However, this efficacy was reduced to 50% at day 33 post-treatment.

In the 1990's, Suphalucksana and Ching, 1991 [21], treated *Haematopinus* spp in buffaloes by injecting ivermectin subcutaneously at a dose of 0.2 mg/Kg, and observed a limited efficacy (reduction of 84%) at day 28 post-treatment, which is contrary to the results reported by Hussain *et al*., in which treatment of *Haematopinus tuberculatus* in buffaloes, using ivermectin at the same dose and administration route, led to and efficacy of 100% at day 28 post-treatment.

Bastianetto *et al*., 2002 [35] reported that abamectin, ivermectin, and doramectin, administered subcutaneously at a dose of 0.2 mg/kg were completely effective at day 2 post-treatment. Similarly, Veneziano *et al*., 2004 [5], reported that eprinomectin – a macrocyclic lactone with zero milk-withdrawal period- used as *pour-on* at a dose of 500 µg/kg body weight, was 100% effective in controlling these lice in buffaloes at day 56 post-treatment.

Our results suggest that the use of both *pour on* fipronil at 1%, and aspersion baths of deltamethrin at 0.1% show comparable efficacy in controlling lice infestation in buffaloes from day 7 to 23 post-treatment. However, fipronil exhibited a greater residual effect in the control of *Haematopinus tuberculatus* in this species, eliminating about 90% of the infestation at day 30 post-treatment, compared to the 2% reduction rate achieved by deltamethrin in our study. Both products are well tolerated by animals, since no adverse skin reactions were observed in response to their topical administration.

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