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Control of *Dermatobia hominis* larvae by manual extraction is effective and does not cause abscess[1]

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ABSTRACT.- Ribas J.L., Veríssimo C.J., Gutmanis G., Andrade M.F., Ribeiro E.G. & Katiki L.M. 2023. **Control of *Dermatobia hominis* larvae by manual extraction is effective and does not cause abscess.** *Pesquisa Veterinária Brasileira 43:e07260, 2023*. Instituto de Zootecnia, Agência Paulista de Tecnologia dos Agronegócios, Secretaria de Agricultura e Abastecimento do Estado de São Paulo, Rua Heitor Penteado 56, Nova Odessa, SP 13380-011, Brazil. E-mail: cecilia.verissimo@sp.gov.br

In order to study alternative ways of control of *Dermatobia hominis* fly larvae, three processes were tested: T1, cypermethrin 5% + *chlorpyrifos* 7% + citronellal 0.5% (Pour on); T2, trichlorfon powder dissolved at 2% in burnt oil passed on the nodules; T3, manual larva extraction. The study was conducted at Instituto de Zootecnia in Nova Odessa, São Paulo State, Brazil. In a dairy herd of 176 animals, 29 with at least one larvae were found, distributed in the three treatments. Each treatment's efficacy percentage was based on the number of live botflies present after 14 days. The animals were observed on days +4, +7, +14 and +21, and new botflies and abscesses were annotated. All treatments presented high effectiveness (above 98%). New botflies were already seen in the first week after applying the three treatments, and on day 21, two abscesses in each chemical treatment were observed. Larva extraction was 100% effective, with no originating abscesses, and should be encouraged in small and medium-sized properties and those with resistant botflies to chemical products.

INDEX TERMS: Alternative control, abscess, botfly, cattle, chemical control, *Dermatobia hominis.*

RESUMO.- [Controle da larva de *Dermatobia hominis* com extração manual é efetivo e não causa abscesso] Com a finalidade de estudar formas alternativas de controle de larvas da mosca *Dermatobia hominis*, foram testados: T1, cipermetrina 5% + clorpirifós 7% + citronelal 0,5% (Pour on); T2, triclorfon em pó, dissolvido a 2% em óleo queimado, passado sobre o nódulo; T3, extração manual das larvas. O estudo foi realizado no Instituto de Zootecnia, localizado em Nova Odessa, Estado de São Paulo, Brasil. Em um rebanho leiteiro com 176 animais, encontramos 29 com, pelo menos, uma larva que foram distribuídos nos três tratamentos. O percentual de eficácia foi baseado no número de bernes vivos após 14 dias da aplicação dos tratamentos. Os animais foram observados nos dias +4, +7, +14 e +21 e foram anotados novos bernes e abcessos. Todos os tratamentos apresentaram alta eficácia (acima de 98%). Logo na primeira semana, foram observados novos bernes em todos os tratamentos, e no 21º dia observamos dois abcessos em cada tratamento químico. A extração da larva foi 100% eficaz, não originando abscessos, devendo ser incentivada nas pequenas e médias propriedades, e naquelas com bernes resistentes a produtos químicos.

TERMOS DE INDEXAÇÃO: Abscesso, berne, controle alternativo, controle químico, *Dermatobia hominis*, gado.

INTRODUCTION

In recent years, researchers have sought sustainable development, and the world is interested in health and environmental concerns, mainly about less contamination by agrochemicals (Azevedo et al. 2011). There is a high demand for organic food, which prompts producers to seek parasite control methods that are environment-friendly and do not compromise meat or milk quality. Controlling the diseases and parasites that cause great losses in cattle farming at the lowest cost, having a more resistant herd and working with a lower risk of environmental contamination are goals of great relevance to cattle farmers (Pinto et al. 2005).

Dermatobia hominis is a fly that lays its eggs on another insect, usually another Diptera, that, by landing on the bovine or other warm-blooded animal, releases the larvae that promptly

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begin their insertion into the subcutaneous tissue. In cattle, the larva goes through three stages, L1, L2 and L3, for 31 to 69 days, depending on the animal (Ribeiro & Oliveira 1983). When the L3 is mature, it spontaneously leaves the host, usually at night (Ribeiro & Oliveira 1983), actively seeking to bury itself in the ground, where it will pupate and turn into a new fly in 26 to 31 days (25ºC, UR 70%) (Barbosa et al. 2002). Its control is usually done with chemicals, and those based on macrocyclic lactones have been used successfully (Roncalli & Benitez Usher 1988, Moya-Borja et al. 1993, Silva Netto et al. 2001); nevertheless, there have been reports of failure in the control of this myiasis with the use of these products (Neves et al. 2015). The nodules of the botfly do not seem to cause much harm from a clinical point of view to the point of impairing the weight gain of the animals that harbor them (Magalhães & Lesskiu 1982, Barbosa et al. 2002). However, the damage is great when the quality of the leather for the industry is taken into consideration (Magalhães & Lesskiu 1982, Marques et al. 2000). In Brazil, the losses caused by botflies have been estimated at more than 380 million dollars annually (Grisi et al. 2014). As the larvae develop, a nodule forms, reaching 2-3cm in diameter at the end of the cycle (Barbosa et al. 2002). In addition, the nodules are usually infected by bacteria (Sancho et al. 1996), some with lethal potential to cattle (Pereira et al. 2000, Ladeira et al. 2010).

The oldest method of *D. hominis* control is the manual extraction of the larvae, used in animals and humans, where they are expelled through the abscess hole. However, only some scientific papers address this natural control (Roncalli 1984, Villarino et al. 2003).

Due to the current need to search for alternatives to control this parasite, the objective of this study was to evaluate the control provided by the extraction of larvae, compared to two other chemical control methods: commercial product in a Pour on formulation and another chemical product in powder form, mixed with burnt oil applied directly to the nodules, both methods widely applied in Brazil.

MATERIALS AND METHODS

The study was carried out with animals from the "Instituto de Zootecnia" dairy herd, a governmental institution of São Paulo State, located in the city of Nova Odessa (latitude 22º46'40" S; length 47º17'45" W), Brazil. The study was approved by the Ethics Committee of this institution (protocol number 281/19). Of a total of 176 animals, 29 with the presence of at least one botfly were distributed in the treatments; the animals were of varying ages, most being adults; the animals' breed varied between pure Holstein and crossbred cattle (Holstein x Jersey x Zebu), with a predominance of Holstein. The assignment lasted 21 days of observation, beginning in March 2019. The herd was meticulously inspected for *Dermatobia* larvae in a cattle crush, where they were immobilized. The treatments were:

T1 – Chemical product in Pour on formula: cypermethrin 5g, chlorpyrifos 7g, and citronellal 0.5g, Colosso Pour on®, N=9;

T2 – Chemical product in powder formula: trichlorfon, Neguvon®, by applying a mixture of 20g of the product in 1L of reused car engine oil directly on each nodule, using a 5cm wide brush, N=10;

T3 – Extraction of the larvae, pressing the nodule, between thumb and index finger, from bottom to top; when the larva was small and difficult to get out, tweezers were used to help in the extraction, N=10.

Five observations were accounted for in the experimental period, i.e., on days 0, +4, +7, +14 and +21 post-treatments. On the day of treatment (day 0), the location of each nodule was marked as a point with a pen on drawings representing the right and left side of the cattle. Then, copies were taken from this original drawing to follow what was happening with the original nodules and to mark the new nodules of botflies noticed on the animals on each evaluation. Subsequently, the location of each nodule was defined according to the figure proposed by Cardoso et al. (2014), which divided the bovine body into seven regions, namely: head, cervical region (neck and shoulder), thoracic limb, rib, flank, pelvic limb, and tail/croup. On each day of observation, the treated nodules were observed for abscess formation or disappearance of the nodule, whether the larva would be dead or alive. Those with an intact orifice through which they breathe were considered alive. Fourteen days after treatment, all the larvae treated on day 0 that could be alive were extracted. To ensure that, they were taken to the laboratory to see normal movements and integrity under a magnifying glass. New nodules with *Dermatobia hominis* larvae that appeared on the fourth day and forward were marked their localization on the cattle drawing on the day they appeared and immediately extracted with the aid of the hands and/or tweezer. No treatment has been done for these new botfly nodules except for manual extraction of the larva.

Extracted larvae were stored in glass vials labeled with the animal number and included in 70% alcohol to evaluate later in the laboratory. With a magnifying glass, the number of respiratory spiracles was evaluated to classify the larval stage: one for the L1 stage, two for the L2 stage, and three for the L3 stage.

The animal's coloration and the nodules' location on the black or white coat (Holstein) and which side they were on, right or left, were also noted.

The formula above was used to evaluate the efficacy of each treatment:

$$\%\text{Efficacy} = \left[\left(\left(\left(\text{Larvae day } 0\right) - \text{Larvae day } 14\right)/\text{Larvae day } 0\right)\right] \times 100$$

After the treatments, each animal remained in its original paddock.

RESULTS

The distribution of botfly nodules in the herd can be seen in Figure 1. The distribution of animals with nodules fits a negative binomial model curve, the majority of the herd (147 animals/84%) was free of nodules of *Dermatobia* larva on day 0, and very few had more than 10 nodules (4/2%); the maximum number of nodules registered in one animal was 17. Considering the 29 parasitized, most (20/69%) had between one and four nodules.

The results of the efficacy of each treatment, new nodules and abscesses observed over 21 days are shown in Table 1. All treatments were highly effective on the larvae (98-100%). New nodules were observed on T1 in the first-week post-treatment; on T2, new nodules were observed in the first-week post-treatment. Regarding T3, the first new nodule was observed on day +4, and it probably went unnoticed on day zero, as it was located on the inner face of the left hand in a place that is difficult to visualize and palpate. All the new botflies (37) were manually extracted once noticed.

Regarding the larval stage, when they were recovered, 76 % were in the L2 stage (two respiratory spiracles) and 24 % in the L3 stage (three respiratory spiracles).

A total of 130 *Dermatobia* nodules were observed on the day of treatment, and 37 more were observed over the 21-day observation period, totaling 167 larvae, 100 on the right side (60%) and 67 on the left side (40%). Table 2 includes the percentage distribution of the larvae in the animal's body, according to body regions (Cardoso et al. 2014).

DISCUSSION

Infestation by *Dermatobia hominis* larvae varies among animals of the same breed, crossbreed, herd and management (Maia & Guimarães 1985, Oliveira & Alencar 1990). This could be verified since only 29 (16%) of 176 animals evaluated on day zero were parasitized, with very few having more than 10 nodules (Fig.1). Further studies with a greater number of parasitized cattle should be made in order to corroborate the results presented in this preliminary descriptive analysis statistically.

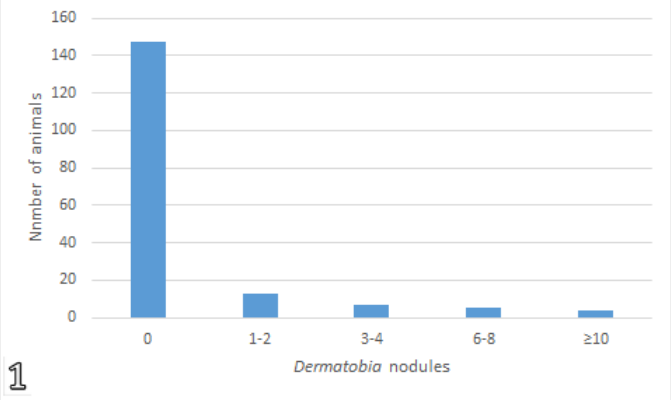

Fig.1. Distribution of animals with 0, 1 to 2, 3 to 4, 6 to 8, and more than 10 nodules of *Dermatobia hominis* on day 0 in which all the herd were evaluated (Nova Odessa/SP, March 19, 2019).

Table 1. Number of *Dermatobia* nodules observed immediately before the application of treatments and 14 days later, efficacy of treatments, total of new *Dermatobia* nodules observed on days 4, 7, 14 and 21 post-treatment, and number of abscesses resulting from dead larvae observed on day 21 post-treatment

	T1	T2	T3
Dermatobia nodules before treatment	49	52	29
Live larva 14 days post-treatment	0	1	0
Treatment efficacy %	100	98	100
New *Dermatobia* nodules	5	22	10
Abscesses	2	2	0

T1 = Pour on product (cypermethrin 5g, chlorpyrifos 7g, and citronellal 0.5g), T2 = Triclorfon (2%) mixed with burnt oil brushed over the nodule, T3 = manual larval extraction.

Table 2. Percentage of larvae found on days 0, 4, 7, 14 and 21 post-treatment in the regions, according to division of the bovine body proposed by Cardoso et al. (2014)

	Head	Neck	Thoracic limb	Ribs	Flank	Pelvic limb	Tail/Rump
%	1.92	7.69	21.15	59.62	3.85	3.85	1.92

In the present study, all treatments were more than 90% effective. However, none of the chemical treatments prevented reinfestation of the animals with new larvae within 21 days. In both chemical treatments, abscess formation was observed; some formed soon after treatment but disappeared over weeks. At 21 days of observation, two nodules with abscesses remained in each of the chemical treatments. Abscess formation was not seen in the treatment with larval extraction, which controlled 100% of the larvae. Larval extraction can be easily done on small and medium farms because not all animals in the herd are parasitized, and most of them have few parasites present in the body (Moya-Borja 2003, Cardoso et al. 2014), which was confirmed in this study. Roncalli (1984) reports that manual extraction is possibly the oldest method of dermatobiosis control. The verification of nodules is performed by visual and tactile inspection (Gomes et al. 1996), and especially the mature larvae (L3) can be easily extracted just by pressing the fingers from below upwards or with the aid of tweezers if they are in younger forms (L2). It is also recommended to eliminate the larvae extracted from the animal to interrupt the biological cycle, and thereby decrease the *D. hominis* fly population at the site. The inspection and extraction of the botflies that parasitize the animals from a herd could be made every 21 days, together with the tick selective control as recommended by Andrade et al. (2022).

Next to the *D. hominis* larvae, many bacteria live in the subcutaneous tissue (Sancho et al. 1996), some very harmful to cattle (Pereira et al. 2000). When the larva dies within the subcutaneous tissue, the orifice, through which it breathes, heals quickly, preventing necrotic material from being expelled naturally, forming abscesses, which, depending on the size they reach, may not be reabsorbed by the body, becoming a problem for the animals. One example is the pathology caused by the bacteria *Mannheimia granulomatis*, one of the bacteria that can be isolated from *D. hominis* nodule (Pereira et al. 2000) and can lead a bovine to death (Ladeira et al. 2010). First-stage larvae were not recovered in this study, probably because they are very small, making them imperceptible on the body.

In this trial, a product based on cypermethrin, chlorpyrifos and citronellal in a Pour on formula had the best protective effect against botflies because this treatment had the lowest number of new nodules besides killed all the larvae that were present in the animals on day 0. Although many dead larvae being expelled by the host were noticed in this treatment, two nodules had not dissipated on day 21 of observation. In 2003, Moya-Borja stated that pyrethroids, commonly used in the control of ticks and flies, had little action against the second and third stages of botflies but were excellent repellents of the eggs' carrier insects and were able to control the early stage of the larva when it penetrates the host's skin. In Brazil, in the 1990s, a study evaluated a pyrethroid-based product (alfamethrin 0.005%) applied as a spray to control botflies, and it was 100 % effective up to 18 days after treatment (Sanavria & Grisi 1991). Kasai et al. (1997) tested the formulation cypermethrin 4.5% + DDVP 50% in the control of *Rhipicephalus microplus* and *Dermatobia* in crossbred animals and found high efficacy for ticks and low efficacy for *Dermatobia* during 42 days of observation; the efficacy for *Dermatobia* ranged from 60% (days 35 and 42) to 72% (21 days after spraying); the explanation for the low efficacy

towards botfly was that the product acted through contact and not through the systemic route, which made it difficult for the product to come into contact with the larvae in the subcutaneous tissue. Valencia et al. (2007), using a spray-on product based on 15% cypermethrin + 25% chlorpyrifos, observed efficacy against botfly of 96.3% (three days after treatment), with a residual effect up to 28 days after treatment (82.7% efficacy on day +28). The combination cypermethrin 15% + chlorpyrifos 30% + fenthion 15% had 100% efficacy on the seventh day after treatment (Corrêa et al. 2012).

Organophosphates are a group of chemical compounds widely used in agriculture and livestock as acaricides and insecticides (Cavaliere et al. 1996). One product from this group (trichlorfon) was studied in a powder form mixed with used oil and brushed onto each botfly nodule. This way of diluting and applying this product with burning oil is very popular among small and medium-sized farmers. Moya-Borja (2003) states that "trichlorfon is the product most used in small properties to control *D. hominis* larvae due to its efficacy and low cost". However, literature on the effectiveness of the treatment with trichlorfon mixed with burnt oil and brushing the nodules is scarce. In a survey, Villarino et al. (2003) found that the most commonly used method of botfly control in Nicaragua was ivermectin (43%), followed by dichlorvos mixed with burnt oil and brushing the nodules (40%) and trichlorfon mixed with burnt oil, also brushing the nodules (19%); only 2% used manual larval extraction. In Brazil, a study of popular knowledge among farmers in the rural vicinity of the city of Formosa, state of Goiás, reports that the medication most commonly used to combat botflies was Neguvon® (trichlorfon) mixed with burning oil (Cansi et al. 2012). The results showed that while achieving 98% efficacy, one larva survived the treatment with trichlorfon mixed burned oil; two abscesses were not dissipated 21 days after treatment. In most of the nodules, the larvae died inside the skin without being expelled, which triggers the inflammatory process in the host.

Until 2003, when Moya-Borja published his considerations on eradicating or integrating the management of neotropical myiasis of the Americas, there were no reports of *D. hominis* resistance to any chemical group of insecticidal drugs. However, drug-resistant parasites are a reality nowadays and a big problem for agriculture and livestock. Many cattle breeders having the empirical perception of loss or decrease in efficacy of insecticides against *D. hominis* larvae and attributing this to the emergence of resistant parasites choose to discontinue the use of certain insecticides in order to employ more broad-spectrum drugs (Oliveira-Sequeira et al. 2014). Regarding injectable endectocidal products, ivermectin and moxidectin, launched in the 1980s and 1990s, respectively (Roncalli & Benitez Usher 1988, Silva Netto et al. 2001), and are still widely used in our environment for botfly control, have already reports of resistance of botfly to these products (Neves et al. 2015). Not to mention that injectable endectocides and other chemical groups in the Pour on formulation cause negative environmental impacts by reducing the coprophagous fauna, which causes a delay in manure decomposition (Bang et al. 2007) and the possibility of inducing resistance to other parasite species, such as nematodes, ticks and other flies, by the amount or by unnecessary exposure to these products (Alegría-López et al. 2015).

Regarding coat coloration, Gomes et al. (1996), evaluating animals of different beef cattle breeds in Mato Grosso do Sul, identified that the infestation was related to the coat coloration of the animals: those with dark coats had more botflies because dark coats attract botfly-vectoring flies more intensely (Guimarães & Papavero 1999). The same was observed by Cardoso et al. (2014), who found that darker animals were more parasitized than lighter ones. This characteristic was noticeable in our study, as most animals were black-and-white Holstein, and 100% of the nodules were in the black coat.

Our data corroborate most of the works which found more nodules in the front region of the body (Pinto et al. 2002, Mozzaquatro & Sanavria 2003, Cardoso et al. 2014): 90% were located in the anterior region, from the rib to the head, since the movements provided by the animal's tail can scare away from the posterior region the insects that could load *D. hominis* eggs (Oliveira 1991). The skin surface of 8,124 cattle slaughtered in a slaughterhouse in the state of Rio de Janeiro over one year was examined by Sanavria et al. (2002), who found a higher incidence in the anterior quadrants, more on the left side and in dark-haired animals; the great majority of the skins (85%) had no lesions caused by *D. hominis* larvae.

In this study, some animals had myiasis caused by *Cochliomyia hominivorax*, but none was directly related to the presence of *Dermatobia*. Maia & Guimarães (1985), observing 10 Nellore cows in a one-year experiment, also found no relationship between these two myiasis but found an association between *Dermatobia* and abscesses, and they cited three other authors who also found this association.

CONCLUSIONS

The mechanical extraction of larvae is an excellent alternative for controlling the dermatobiosis caused by *Dermatobia hominis,* especially in small and medium farms. Although it requires more attention and labor, it does not need any products to control, bringing savings to the production system. In addition, using this method reduces the likelihood of developing resistance to ectoparasites. It prevents abscess formation, contamination of the environment and the animals and their products. Also, it is perfect for organic production systems and farms where chemical products are no longer effective against this parasite.

It is recommended that the larvae removed from the animals be destroyed appropriately, so they cannot complete the parasitic cycle, thus reducing the population of botflies on the property.

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