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Short Communication

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First Report on the Use of Larvae of *Cochliomyia macellaria* (Diptera: Calliphoridae) for Wound Treatment in Veterinary Practice

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Abstract

Factors contributing to the delay or prevention of the natural wound healing process include infection and ineffective conventional treatment. Alternative therapies, such as the maggot debridement therapy (MDT), may be helpful for successful treatment in these cases. Aiming to disseminate information about the possibility of using other species of flies for wound treatment, besides the best known *Lucilia sericata* Meigen, 1826 (Diptera, Calliphoridae), we report here a successful MDT case with the application of *Cochliomyia macellaria* (Fabricius, 1775) larvae to treat an infected wound with extensive area of necrotic tissue in a dog. Five sterile larvae were applied to each square-cm of lesion and kept on the animal for only 48 h. The healing was successful, from both qualitative and quantitative points of view. The ratio of wound healing (RWH) reached almost 50% on the 5th day and 100% on the 14th day after MDT. Although the overall animal prognosis had been unfavorable, mainly due to the sepsis, the patient began to recover and had improved clinical condition from the fifth day after MDT. This study shows the importance and effectiveness of MDT in promoting faster and more complete healing of a complex wound.

Key Words: maggot debridement therapy, blow fly, infection, healing, alternative treatment

Skin, subcutaneous, and underlying muscle wounds are among the most common injuries treated by veterinarians. In general, these wounds may be caused by bites, car accidents, laceration by sharp objects, or burn injuries. Most often, wounds heal without complications following basic treatment approaches, which incorporate principles of aseptic technique with effective tissue management through surgical, enzymatic, or autolytic debridement (Howe et al. 2015). Other methods used for treatment include the application of growth factors, the use of dressings, plant-derived (e.g., chitosan, sugar) or animal-derived products (e.g., honey/propolis), and ozone and negative pressure therapies (Senel and McClure 2004, Carnwath et al. 2014).

Extensive wounds and/or those that do not heal completely after conventional or even surgical treatment represent a major problem for animal health and welfare. In addition to pain, such wounds may also lead to localized infection, sepsis, and amputations, and loss of use of the animal as well as treatment may incur significant costs (Rey et al. 2010).

Maggot debridement therapy (MDT) is a treatment that uses disinfected necrophagous fly larvae to promote healing of wounds. It may be a good alternative to achieve a successful outcome in cases where complications are observed. Unlike other methods such as enzymatic debridement, which is not recommended for larger injuries, or autolytic debridement, which is not indicated for areas with large amounts of necrotic tissue (Howe et al. 2015), MDT can be used, and should be preferred, when treating extensive or infected wounds with large amounts of devitalized tissue (Sherman et al. 2007).

MDT effects and benefits have been extensively documented (Van der Plas et al. 2009, Masiero et al. 2017, Tamura et al. 2017); these effects include debridement, disinfection (from excreted/secreted substances by larvae with antimicrobial potential), inhibition of the inflammatory process, and stimulation of angiogenesis and of the healing process.

Accumulated evidence in human medicine has suggested the value of the use of the MDT also in veterinary medicine. The green bottle fly, *Lucilia sericata* Meigen, 1862, is common throughout the temperate regions of the world (Rognes 1991), and is the most well-known dipteran with regard to MDT (Stadler 2019). In Brazil, its distribution is restricted to the southern or high altitude wild areas.

Therefore, we have conducted studies using the Neotropical necrophagous species *Cochliomyia macellaria* (Fabricius, 1775) (Dear 1985). The results thus obtained show that the application of larvae of this species on injuries represent a viable alternative for the treatment of wounds from any etiology (Nassu and Thyssen 2015), including those complex or infected (Masiero et al. 2017). In addition, *C. macellaria* is easy to maintain for many generations under laboratory conditions (Nassu and Thyssen 2015), a key feature for larval mass production for medicinal purposes (Stadler 2019).

With the aim of disseminating information about the possibility of using other species of flies—besides the already known green bottle fly—for wound treatment, thereby addressing the regional demands of human and veterinary medicine, we report here a successful MDT case with the application of *C. macellaria* larvae to treat an infected wound with extensive area of necrotic tissue in a dog.

Materials and Methods

Patient's medical history

One male dog (German Shepherd breed, 2 yr old, weighing 35 kg) was admitted to the Hospital of Clinical Veterinary of the Federal University of Pelotas, State of Rio Grande do Sul, Brazil. The animal had a traumatic lacerative lesion in the right pelvic limb. According to the owner, the wound resulted from other dogs' bites. On admission, the patient had significant blood loss due to lacerations in large vessels. Erythrogram yielded the following results: blood cells 6.33 M/mm^3 (normal range: 6–8), mean corpuscular volume (MCV) 66.0 (normal range: 60–77), hemoglobin 13.7 g/dl (normal range: 13–18), hematocrit 41.8% (normal range: 37–55), and red blood cell distribution width (RDW) 10.6% (normal range: 12–15).

On the same day of the hospital admission, the patient was taken to surgery and, given the severity of the injury, the right posterior limb was amputated. Analgesic and antibiotic therapy (metronidazole and ceftriaxone) were administered systemically and the patient was maintained with a surgical drain. About 24 h after the surgical procedure, a postoperative complication resulted in the dehiscence of the lesion. The wound had necrotic tissue, purulent exudates in large quantity, and edema of the peripheral tissue was also observed. Subcutaneous tissue injury reached the muscle fascia. After 8 d, the overall animal prognosis was unfavorable due to the presence of infection, circulatory deficiency, anemia, renal failure, and sepsis. On the 10th day after hospital admission, MDT was applied. The patient remained in the hospital for another 13 d.

The care and comfort of the animal were monitored under guideline of the Ethics Committee on Animal Experimentation of Department of Microbiology and Parasitology, UFPel (#3600/15).

Source of medical maggots and MDT

Colonies of *C. macellaria* were established from adult flies collected in the field (São Paulo State, Brazil, 22°54′21″S, 47°03′39″W). The collected specimens were taken to the laboratory alive, temporarily anesthetized by freezing at −20°C for approximately 3 min, identified, and transferred to screened plastic cages in a climate-controlled room at 27 ± 1°C, 70 ± 10% relative humidity, and a 12-h photoperiod. Adult flies were provided water and sugar ad libitum. A portion of raw ground beef was used to stimulate oviposition.

With the aid of a brush, the eggs were removed from the substrate, washed for 3 min with 1% sodium hypochlorite (NaClO) for disinfection [as proposed by Thyssen et al. (2013)] and kept on moistened filter paper in a climatic chamber (Model 202/4, Eletrolab, São Paulo, SP) at 25°C for 16 h, until the larvae hatched. The resulting larvae (at the beginning of the second instar) were then used in the treatment.

To confirm the disinfection before application, 1 ml aliquots of water used in the last rinse of the eggs were inoculated by seeding in Petri dishes with the following culture mediums: brain heart infusion (BHI), blood agar, and Sabouraud dextrose agar (SDA). The plates were incubated in a bacteriological incubator at 37°C and the results were read at 24 and 48 h.

Five sterile larvae were applied to each square-cm of lesion, as recommended by Nassu and Thyssen (2015). During treatment, the wound was covered with polyurethane dressing, sterile gauze, and tape. The treatment lasted 48 h and the wound was evaluated until complete healing. No other treatment was used after removal of the larvae.

Wound assessment

To qualitatively assess the injury and the healing process, the following parameters were observed: shape, aspect of edge, depth, quantity of necrotic tissue, type and quantity of exudate, and type of granulation tissue (Table 1).

Furthermore, photographic records were taken before, during and after the treatment was performed, to document the results and to assess the ratio of wound healing (RWH).

RWH represents the reduction percentage of the wound in relation to its size before the beginning of the treatment. It is calculated by the formula:

$$\mathrm{RWH} = \frac{A\,(i) - A\,(f)}{A\,(i)} \times 100$$

where $A(i)$ = wound area at day zero, i.e., before treatment; $A(f)$ = wound area on the day of evaluation. Areas were calculated using software ZEN/Zeiss (Germany) version 2.0. Reported values represent the percentage (%) of wound healing.

Results

Immediately after larvae removal, it was remarkably observed that the lesion contained no necrotic tissue nor purulent exudate anymore

Table 1. Evolution of the wound healing process in a dog from the evaluation of parameters, such as shape, aspect of edge, depth, quantity of necrotic tissue, type and quantity of exudate, and type of granulation tissue, before and up to 14 d after the MDT

Evaluated parameters[a]	Before the treatment	Days after the treatment				
		1st	3rd	5th	7th	14th
Shape	I	I	I	I	I	I
Edge	I	I	I	I	II	II
Depth	I	I	I	I	I	II
Quantity of necrotic tissue	I	II	II	II	II	II
Type of exudate	I	II	III	III	III	III
Quantity of exudate	I	II	III	III	III	III
Type of granulation tissue	I	II	III	III	III	IV

[a]Parameters for: *Shape:* I = irregular, II = regular; *Edge:* I = well defined, not adhered to the base of the wound, II = defined, visible contour, adhered to the base of the wound; *Depth:* I = total loss of skin involving subcutaneous tissue damage extending to muscular fascia, II = erythema on whole skin; *Quantity of necrotic tissue:* I = abundant; II = absent; *Type of exudate:* I = purulent, II = aqueous, III = absent; *Quantity of exudate:* I = moderate, II = minimum, III = absent; *Type of granulation tissue:* I = absent, II = roseate, III = bright red, IV = intact skin.

(Fig. 1). In addition, odor and phlogistic signs present before MDT were then significantly reduced.

The healing was successful from both qualitative (Table 1) and quantitative (Fig. 2) points of view. The presence of granulation tissue throughout the wound area could be observed (Fig. 1). The RWH reached almost 50% on the 5th day and 100% on the 14th day after MDT.

Larvae recovered after MDT were alive and active showing that systemic medications (antimicrobial, anti-inflammatory, and analgesic) administered to the patient before this treatment did not affect their viability.

The patient showed no behavioral changes throughout the treatment. Additionally, no adverse or side effects (such as bleeding or pain) were observed. Recovery and stability of the patient's general clinical condition were clearly observed from the fifth day after MDT.

Discussion

Although there are reports of fly larvae use to treat wounds in a reasonable number of animals, such as dogs, cats, rabbits, rodents, buffaloes, cattle, and horses (Sherman et al. 2007, Jones and Wall 2008, Rey et al. 2010), the MDT is still unusual in veterinary practice. As in the case of human medicine, MDT has been most commonly applied in the treatment of traumatic and pressure wounds—in particular for small animals—for the reduction of malignant and benign tumors or the elimination of abscesses, as well as the management of chronic wounds, infected or not by multiresistant microorganisms (Rey et al. 2010). Our results reinforce that MDT is a promising and safe-to-use therapy also for the treatment of complex wounds, even to the point of eliminating the need for additional surgical interventions. In general, surgical debridement is selected as the first line of treatment; however, it is not a conservative procedure. All adjacent tissues, besides those affected, are removed, and usually granulation tissue formation is inhibited (Holt and Griffin 2000). It is also important to emphasize that, unlike surgical debridement, with MDT the living tissue remains intact.

Wounds caused by bites are often densely colonized by bacteria and other microorganisms. They may proliferate rapidly and infect adjacent tissues and the bloodstream; in some cases, this results in sepsis (Holt and Griffin 2000), as seen in the present case. For this type of wound, the elimination of all infected and necrotic tissues is essential for successful treatment and thus to patient recovery. In this respect, MDT achieved the desired goal in a short time. Bohling et al. (2004) recorded an RWH of 43.1 and 76.1% on the 7th and 14th days, respectively, after a surgical procedure in dogs; a value close to 100% was recorded only 21 d later. In contrast, with MDT we found an RWH of 100% on the 14th day.

Antibiotic and anti-inflammatory therapies were ineffective in promoting disinfection and healing, demonstrating that the results obtained by MDT may be more promising than conventional therapies.

Due to the patient clinical condition, surgical debridement could not be performed. Unfortunately, in veterinary practice, MDT has been the last resort for wound care, usually after all other alternatives have been exhausted (Sherman et al. 2007). This is due to some misconceptions and lack of knowledge about this alternative. Larvae of some fly species such as *Cochliomyia hominivorax* (Coquerel, 1858) in the New World and *Chrysomya bezziana* Villeneuve in the Old World cause obligatory myiasis resulting in economic losses and high mortality rates (Hall and Wall 1995). MDT is a particular type of myiasis in which controlled conditions guarantee safety and therapeutic efficacy. In this study, it was possible to report the selectivity of *C. macellaria* larvae in removing only necrotic tissue and wound exudates, a behavior quite different from that observed for obligatory myiasis-causing flies.

In the case reported here, just a 48 h application of MDT was sufficient to remove all necrotic tissue and purulent exudate. According to Blake et al. (2007), approximately 100 larvae within 48 h can debride 50 g of devitalized tissue. The number of larvae to be applied is determined by the sizes of the wound and of the animal. At least one report recommends that large wounds in small animals should be treated with lower larval concentrations (Jones and Wall 2008). The period during which the larvae can remain in the wound bed may

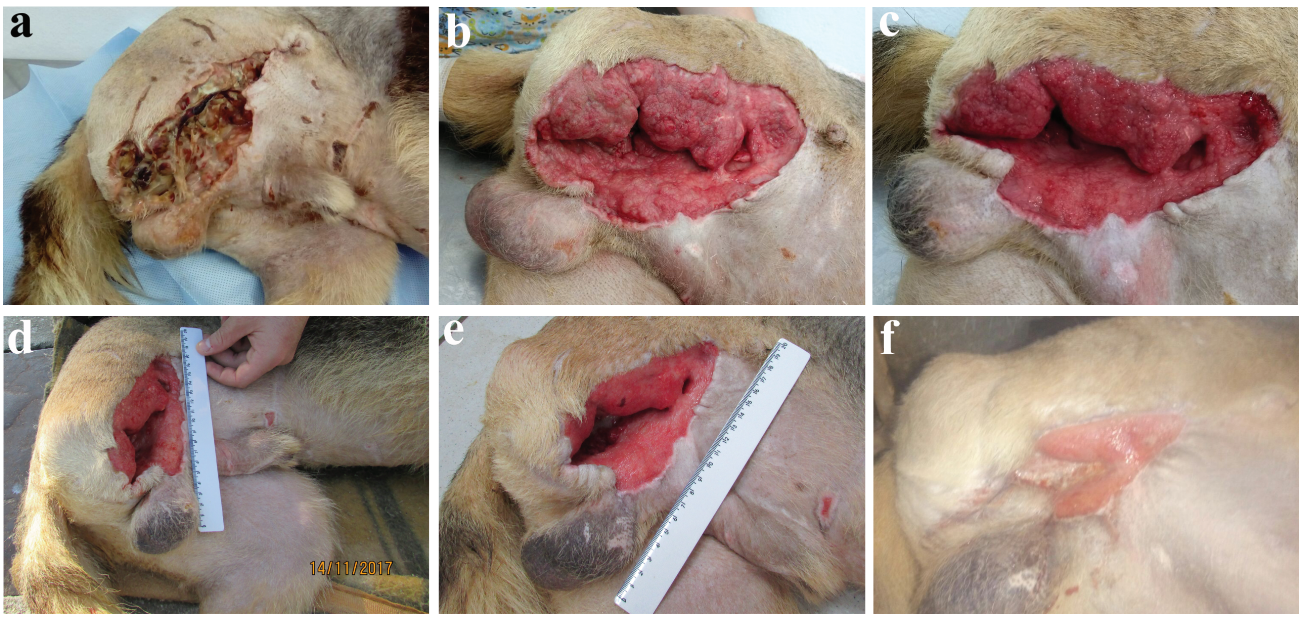

Fig. 1. Evolution of the wound healing process in a dog before and up to 14 d after the MDT. In: A = before MDT; B–F = 1st, 3rd, 5th, 7th, and 14th days after MDT, respectively.

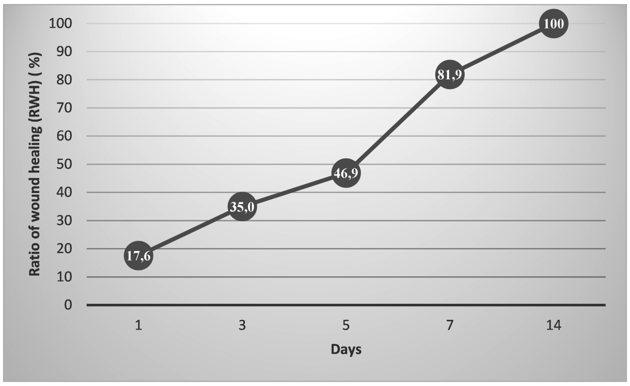

Fig. 2. Ratio of wound healing (RWH; %) on the 1st, 3rd, 5th, 7th, and 14th days after MDT.

vary from case to case and can reach a maximum of 72 h (Nassu and Thyssen 2015). Longer periods are more favorable because they reduce the frequency of dressing changes, but certain precautions should be taken to provide sufficient oxygen for the survival of the larvae, as well as not to drown them, in situations where the wounds are very wet.

This is the first public report of the use of MDT by *C. macellaria* in veterinary practice, and the results are clearly similar to those documented for *L. sericata* (e.g., Sherman et al. 2007). Therefore, the mechanisms by which larvae promote healing are probably the same for both blow fly species: they release proteolytic enzymes (trypsin, chymotrypsin, and collagenase) that digest devitalized tissue; through their secretions, they stimulate the release of growth factors, which act in the formation of healthy tissue (granulation); also, their secretions act on the host's immune system, preventing the constant release of inflammatory cytokines that could impair tissue regeneration (Van der Plas et al. 2009, Tamura et al. 2017). In the same way, the known and documented fight against microbial infection by fatty acids, peptides, and other substances present in larval secretions should also be similar between the two species (Bexfield et al. 2004).

Some practical issues related to the use of MDT—such as appropriate techniques for retention of larvae in the wound bed, correct amount of larvae to be applied, doubts about concomitant use of conventional and traditional therapies—can be easily overcome by taking certain precautions and guidelines into account. However, logistical issues regarding the transport and reception of larvae at the application site for therapeutic purposes (Stadler 2019) can be a challenge and must be properly addressed, since the production of the larvae happens in a separate and usually distant facility. The current study showed that other species of blow flies, besides *L. sericata*, can be potentially important worldwide for MDT use. Some advantages of the use of *C. macellaria* that could be pointed out in this study include: its wide distribution in the Neotropical region, the possibility of mass rearing, and the similarity of mechanisms of action of their larvae on the wound healing process to those of other therapeutic flies.

The unavailability of larvae for wound care represents a major barrier to the large-scale MDT use. Thus, the use of local species, as seen here, can be a key factor in overcoming this issue.

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