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A positive association of larval therapy and hyperbaric oxygen therapy in veterinary wound care

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ABSTRACT

The treatment of cutaneous wounds is part of the veterinary routine from initial scientific reports due to being regularly present condition. Currently, several types of treatments are available to accelerate the healing process. This report presents the case of a dog with multiple lesions in the thoracic limbs resulting from a car accident, who underwent larval therapy and hyperbaric oxygen therapy (HBOT). The animal was a 2-year-old female mixed breed dog presenting severe skin degloving, fracture in the left thoracic limb (LTL), with abrasion lesions and dislocation in the right thoracic limb (RTL). The animal underwent multiple modality therapies, such as HBOT sessions associated with larval therapy; even after the LTL presented gangrene, this treatment resulted in optimal viability of the non-necrotic tissue adjacent to the gangrene. Due to chronic pain unresponsive to drug control and the presence of a fracture at a location where a possible exoprosthesis was supposed to be fixed, the LTL ended up being amputated. There are several reports of the use of HBOT or larval therapy in traumatized limbs; however, the combination of both therapies has not been previously described in the veterinary literature. Thus, we demonstrate through this report that it was possible to quickly recover the animal with good wound resolution through tissue oxygenation and a healthy granulation bed, both provided by the therapeutic combination.

1. Case report

Car accidents involving dogs represent 30%–91% of the cases of blunt trauma treated in veterinary clinics [1]. In most cases, these accidents result in the lesions in the skin, subcutaneous tissue, muscles, tendons, vessels, and peripheral nerves, often associated with limb fracture [2]. The presence of multiple trauma indicates an unfavorable prognosis for the animals that are run over [1], which justifies a rapid medical intervention in these cases. The wounds can be treated using several approaches, always seeking healing and rapid recovery [3].

Veterinary Hyperbaric Oxygen Therapy (HBOT) is highlighted in this area, presenting benefits in the treatment of several types of lesions. The patient is placed in a chamber under pressurized 100% oxygen [4]. The high pressure dissolves the oxygen in the plasma and increases oxygenation in various tissues of the body, thus not requiring oxygen from hemoglobin to ischemic areas or in the case of hypoxia from injuries [4,5].

Larval therapy promotes tissue debridement, reduces the number of microorganisms, and stimulates the formation of granulation tissue in the contaminated wounds [6]. The therapy consists of using live sterile fly larvae (Diptera: Calliphoridae) obtained from a laboratory to treat the lesions, particularly chronic or infected wounds [7].

A 2-year-old female mixed breed dog weighing 4.6 kg was referred to the University Veterinary Hospital of the Federal University of Santa Maria, Santa Maria, Brazil after being run over by a car. According to the clinical history, the traumatic event had occurred more than 24 h back,

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without veterinary assistance or medication administration. Physical examination showed lesions in both thoracic limbs (TLs), with the left thoracic limb (LTL) severely injured and presenting traumatic skin degloving and active bleeding. The dog could not bear weight on both TLs.

The animal was immediately referred to the operating room for lesion debridement and cleaning and to have the bleeding wounds sutured (Fig. 1A). After stabilization, radiographic examination showed a simple complete open transverse fracture of the middle-third of the radius diaphysis on the LTL and radius carpal dislocation on the RTL (Fig. 1B). The fracture was treated using closed reduction with a type 2 external skeletal fixator using 2 mm Steinmann pins and polymethylmethacrylate resin 2 days after diagnosis, in a new surgical intervention (Fig. 1C). The dislocation was reduced and stabilized by external coaptation using an aluminum splint molded exclusively for the dog.

Due to the severity of the lesions and the possibility of LTL gangrene, an HBOT protocol was initiated on the second day of hospitalization, consisting of a 45-min daily session under 2.5 atm absolute (ATA) of pressure (1.5 ATA from the hyperbaric chamber plus 1 ATA from the environment). The dog was prepared for the HBOT sessions by removing metallic objects from her body (except for the external skeletal fixator that was wrapped in gauze and bandages with no addition of chemicals) and having her hair humidified, minimizing static electricity. Soon after, the dog was placed in the hyperbaric chamber for animals (HVM model H1 - veterinary hyperbaric chamber) (Fig. 2A), which was pressurized following the previously stipulated protocol, with 15 min until the desired pressure was reached in stages (one-third of the therapy pressure every 5 min). Once the therapy pressure was reached, the patient remained inside for the stipulated time (45 min), with the pressure being also decreased in stages for 15 min until equalization with the ambient atmospheric pressure. After ten HBOT sessions, the LTL showed gangrene immediately distal to the external skeletal fixator and without evidence of proprio- or nociception (Fig. 1D).

It was decided to initiate larval therapy on the gangrenous tissue. In a laboratory environment, *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) eggs were disinfected using 0.5% sodium hypochlorite for 5 min under constant agitation and then washed thrice using 0.9% NaCl. To confirm disinfection, the eggs were inoculated in plates containing MacConkey agar and incubated in a bacteriological oven at 37 °C for 24 h.

The first larval therapy session was performed with first instars (Fig. 2B) to debride the wound on treatment day 12, in which, after applying the larvae on the lesion, a dressing was performed with the aid of sterile gauze and bandages all over the limb to prevent the invasion of other insects and the applied larvae from escape and leaving the wound. After approximately 48 h, third instars were removed due to advancing larval development, and the animal underwent another HBOT session. Soon after, first instars were placed on the wound for the second larval therapy session (treatment day 14). On the fourth day of larval therapy (treatment day 16), there was extensive cleaning of the gangrenous area, leaving only macroscopically healthy bone and muscle tissue (Fig. 2C). It is important to note that the HBOT sessions were suspended during larval therapy, being subsequently resumed, as there are still no data on how HBOT could act on larvae (For a better understanding, Table 1 shows the therapeutic protocol used). After checking if there was bone contamination, the external skeletal fixator and the bone fragments distal to the fracture line were removed with the patient under sedation and analgesia. The remaining short bone fragments (a proximal third of radius and ulna) would hinder the installation of an osseointegrated exoprosthesis. Additionally, since the animal started presenting chronic pain unresponsive to clinical treatment, the LTL was amputated. The surgical amputation was performed after 17 HBOT sessions, using the technique described by Schulz et al. [8] with some modifications, with scapular neck and acromion section.

The tissues adjacent to the traumatic lesions and gangrenous area in the LTL showed excellent viability, including rapid control of ascending infection with the combination of larval therapy and HBOT (Fig. 2D). The authors believe that the combination of HBOT and larval therapy ensured blood support to the viable tissue and the removal of necrotic tissue, allowing rapid recovery of the affected area.

Veterinary care must be quick and efficient in multi-traumatized animals due to the high mortality rates of patients severely hurt (12%–14%). These rates are underestimated since most animals die at

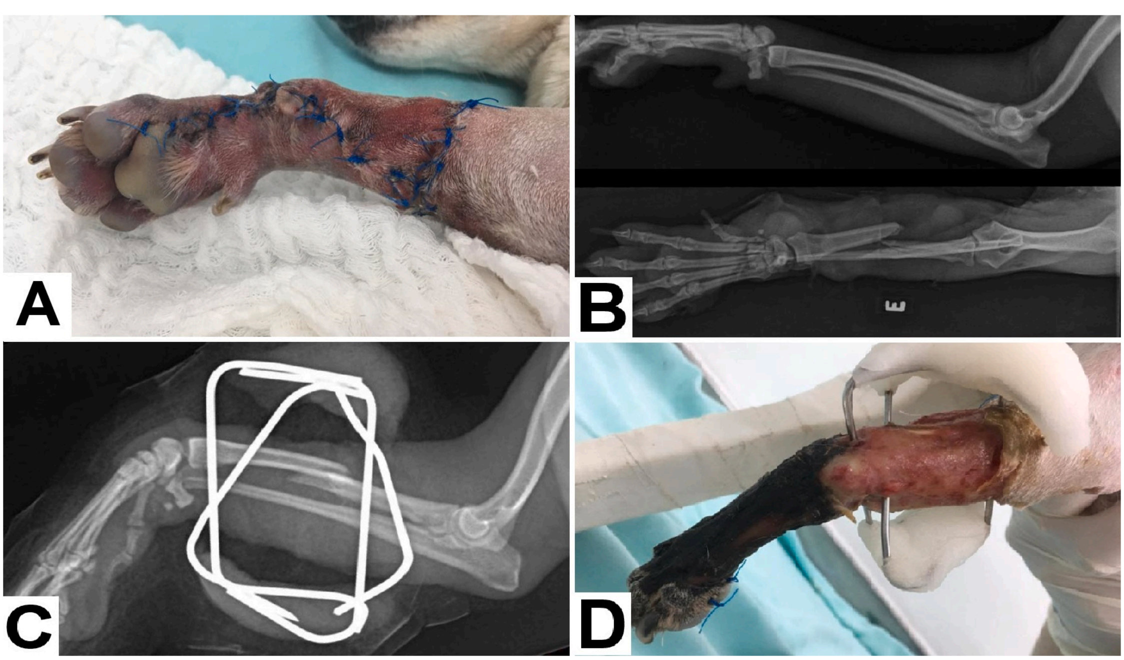

Fig. 1. The left thoracic limb (LTL) immediately after surgery to treat wounds and bleeding (A). Radiographic image of the TLs showing radiocarpal dislocation and radius diaphysis fracture (right thoracic limb [RTL] and LTL, respectively) (B). Radiographic image of the LTL after orthopedic surgery (C). The LTL after 10 hyperbaric oxygen therapy (HBOT) sessions (12 days after the accident) showing the presence of gangrene immediately distal to the external skeletal fixator (D). Source: Personal archive.

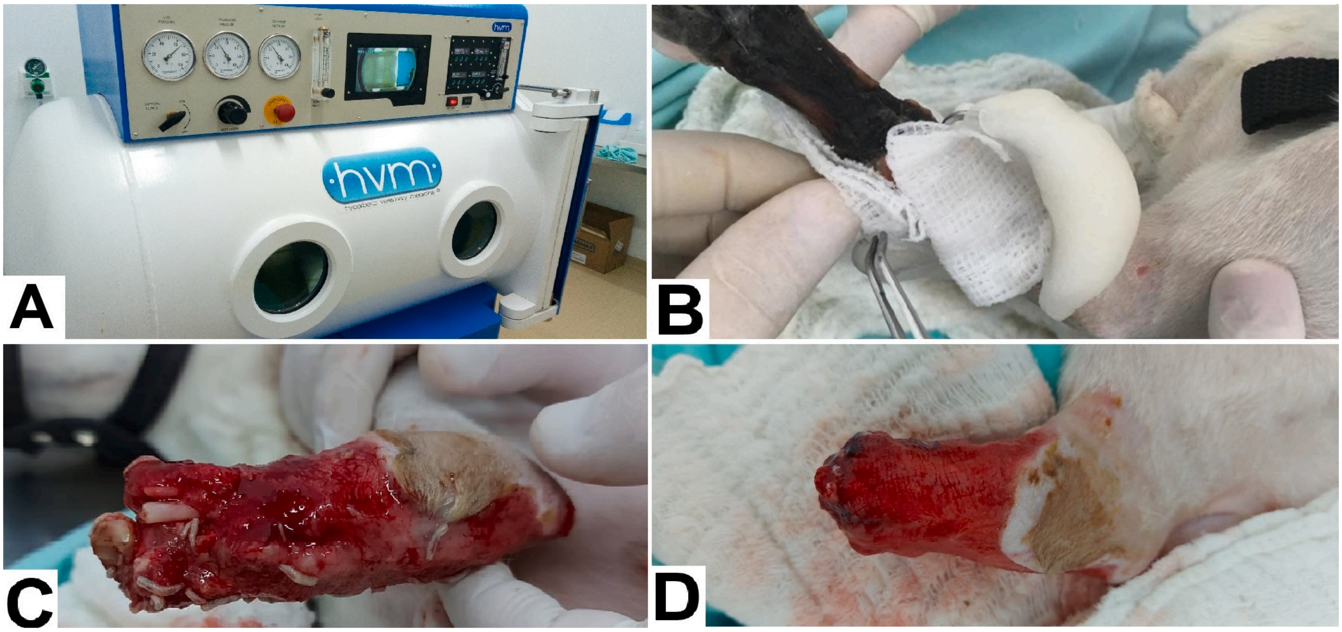

Fig. 2. Specific hyperbaric chamber for veterinary use at the Federal University of Santa Maria (HVM model H1 - Veterinary Hyperbaric Chamber) (A). The left thoracic limb (LTL) during larval biotherapy (B). The LTL during larva removal (second session) (C). The LTL immediately after the last hyperbaric oxygen theory (HBOT) session showing a healthy granulation bed (D). Source: Personal archive.

Table 1
Therapeutic protocol used per day of hospitalization.

Procedure	Hospitalization Day					
	1	2–12	12–14	14–16	17–20	21
Hospitalization, exams and first support	X	–	–	–	–	–
HBOT[1]	–	X	[3]	[3]	X	X
First Larval Therapy Application[2]	–	–	X	–	–	–
Second Larval Therapy Application[2]	–	–	–	X	–	–
Amputation and Hospital Discharge	–	–	–	–	–	X[4]

[1] 45-min daily session under 2.5 atm absolute (ATA) of pressure (1.5 ATA from the hyperbaric chamber plus 1 ATA from the environment).
[2] larval therapy using first instars of *Lucilia cuprina* Wiedemann (Diptera: Calliphoridae) (third instar removed after approximately 48 h).
[3] HBOT performed before the application of larval therapy and after removal, never together.
[4] Procedure performed immediately after HBOT.

the accident site, and are therefore, not accounted for [9]. HBOT may bring several benefits to the run-over patient, which is indicated in cases of crush injuries, compartment syndrome, acute traumatic ischemia, and severe blood loss. Its action against necrotizing soft tissue infections, thermal burns, complicated wound healing, osteomyelitis (refractory), grafts, and skin flaps, among others, have been highlighted in scientific reports [4,10]. These conditions justify the choice of HBOT for the reported animal.

There are reports of the use of 2.5 ATAs as therapeutic pressure for cases with skin lesions [11], where HBOT can increase fibroblast proliferation and collagen deposition, decrease edema by vasoconstriction, increase antimicrobial activity, and modulate the immune system and angiogenesis [10,12]. In the reported case, the use of 2.5 ATAs corroborated the literature, since the presence of exudate and hyperemia near the wound soon after the HBOT sessions suggested a favorable environment for antimicrobial and angiogenic actions. Even though HBOT showed no action on the gangrene, the progressive improvement of adjacent lesions was noticeable after each session.

Oxygen is necessary to the body as it is directly linked to energy supply and cellular respiration. In case of tissue injury, the oxygenation capacity of the injured tissue is reduced, increasing the time to deliver oxygen to that location [5]. HBOT is contraindicated for animals with pneumothorax or pneumomediastinum; in coma, unconscious, or semiconscious; and respiratory insufficiency [4]. As long as these restrictions are followed by the veterinarians and the multi-traumatized patient is conscious, there are no impediments for the HBOT sessions.

Another recommendation is to pressurize and depressurize the chamber in stages to avoid side effects, such as oxygen-induced convulsion and auditory and pulmonary barotrauma [4,10]. In the reported hospital, each step is standardized to take 15 min to maximize patient comfort and safety in both steps.

Larval therapy has been reported in several animals, such as dogs and cats, with successful wound healing without adverse consequence [13]. It is also used to treat abscesses, some types of malignant and benign tumors, and chronic wounds [14]. However, the greatest challenge reported by veterinarians was to retain the larvae in the wounds [15], which was not observed in this case due to the use of occlusive dressings (already mentioned) to keep the larvae for a predetermined period, considering the biology of L. *cuprina* and time needed to reach the thrid instar.

In this wounded dog, larval therapy was chosen considering the following: the extensive and deep area of tissue necrosis that extended to bone tissue, making outpatient surgical debridement unfeasible; requirement of general anesthesia for a possibly long procedure; and that the animal did not present clinical conditions to undergo a surgical procedure at that time.

In a previous case report [16], the researchers used larval therapy in a male traumatized dog that came to the clinic. There was deep and extensive laceration with tendon, muscle, and bone exposure and rupture in the LTL caused because of the car accident. Despite the severity, the edema, foul odor, and inflammation were reduced on the third day after treatment, with increased granulation tissue and blood irrigation, resulting in excellent healing [16]. These findings corroborate our study, in which the animal presented evident blood support and extensive necrotic tissue removal on the fourth day after larval therapy.

Larval therapy is a safe and effective treatment for wounds, associated with three broad actions: debridement, disinfection and accelerated tissue growth, which presents a large number of interactions between the larva and the host, both physical and chemical [17]. Additionally, larvae excrete ammonium bicarbonate and its derivatives into the environment, neutralizing the acid exudate produced by wound inflammation, increasing the pH, and reducing bacterial colonization [18]. The reported animal showed no discomfort with the presence of larvae in the wound, with extensive cleaning of the necrotic area at the end of the first application of larvae.

There are few studies on the use of larval therapy and HBOT in veterinary medicine; therefore, further veterinary studies are necessary to validate this combination, mainly due to the growing bacterial resistance to current drugs [5,19].

The present report presents the possibility of combining larval therapy and HBOT to optimize the therapeutic time, control infection, and remove extensively necrotic tissues, provided that the indications and contraindications of each of these therapies are observed.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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