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Dog bite trauma resolution in tortoise (*Chelonoidis chilensis*) by use of propolis tincture

Resolución de traumatismo por mordedura de perro en tortuga chaqueña (*Chelonoidis chilensis*) utilizando tintura de propóleo

Vetter-Hiebert Joerg Richard*, Pintos-Casamayouret Sergio Rafael

Article Data

National University of Asunción.
Department of Faunal Resources and Natural Environment.
Faculty of Veterinary Sciences.
Ruta PY02 km 13,5.
San Lorenzo.
Tel: +595 983 944 299.

***Contact address:**

National University of Asunción.
Department of Faunal Resources and Natural Environment.
Faculty of Veterinary Sciences.
Ruta PY02 km 13,5.
San Lorenzo.
Tel: +595 983 944 299.

Joerg Richard Vetter-Hiebert
E-mail address: jvetter@vet.una.py

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Abstract

The case of two Chaco tortoises, *Chelonoidis chilensis*, attacked by canines is described. The patients presented fractures of the carapace in the caudal portion, involving loss of bone tissue, with exposure of connective tissue. The treatment consisted in hooks that were attached to the shell using epoxy resin, bringing the skin close to them with 0.30 mm polyamide, combined with cefotaxime, and tramadol and meloxicam as analgesic. The open spaces between skin and shell were treated with a combination of chlorhexidine with glycerin, and covered with 20 % propolis tincture. The patients were kept in a clean environment, but without manipulation of atmospheric variables. Sutures were removed after 5 weeks, after which the adhesion of the integument and the stability of the wounds were verified.

Resumen

Se describe los casos de 2 ejemplares de tortuga chaqueña, *Chelonoidis chilensis*, atacadas por caninos domésticos. Los pacientes presentaban fracturas del caparazón en la porción caudal, involucrando la pérdida de tejido óseo, con exposición de tejido conectivo. El tratamiento consistió en la fijación de ganchos al caparazón mediante resina epoxi, aproximando la piel a los mismos con poliamida 0.30 mm, acompañado de la administración de cefotaxima, y como analgésico el tramadol y meloxicam. Los espacios abiertos entre piel y caparazón fueron tratados con una combinación de clorhexidina con glicerina, y cubiertos con tintura de propóleo al 20 %. Los pacientes fueron mantenidos en un ambiente limpio, pero sin manipulación de las variables atmosféricas. A las 5 semanas se retiraron las suturas y se constató la adhesión del tegumento y estabilidad de las heridas

Introduction

The Chaco tortoise, *Chelonoidis chilensis* (Gray, 1870), is a terrestrial species widely distributed in Argentine and Paraguayan territory, as well as in southern Bolivia[1]. Its shell, divided into shields, called vertebral (in number of 5), costals (4 pairs), and marginals (11 pairs)[2].

Tortoises frequently present lesions on their shell, for various reasons, such as lawnmowers, vehicles, or bites from predators, whether they are dogs, foxes, or rats[3-6]. In cases of dog bites, the clinical presentation can be diverse, ranging from abrasions on the shield, to cracks, compression fractures, or the loss of entire parts of the shell, such as its margins, exposing bone surfaces and even viscera[7].

In cases involving loss of tissue, which produced the separation of the skin and carapace, carapace suture was used successfully, using attached hooks[7,8].

Propolis, a natural resin of vegetable origin, collected by honey bees (*Apis mellifera*) as a construction and protection material for the hive, used for thousands of years in traditional medicine, with antibacterial and antifungal activity, preventing and inhibiting the proliferation of pathogens[9]. Used during the proliferative phase of healing, it not only has anti-inflammatory effects, but also increases the proliferation, activation and synthesis capacity of fibroblasts, thus accelerating the healing process[10].

Propolis reduces the acute inflammatory response, a phase in which there are neutrophils at the lesion site, which in large numbers can cause necrosis and tissue damage, which in turn delays the healing process[10]. By reducing the number of neutrophils at the injured site, fibroblast proliferation begins earlier, allowing more accelerated epithelialization[10]. Comparing the antibacterial, anti-inflammatory and antioxidant effects obtained with the use of propolis in in-vitro studies, the same effects are not always achieved *in-vivo*, due to the anatomical and physiological differences between different animal species, they are not always considered when trying to reproduce the findings of the study in a clinical setting. Reports on the use of propolis for the treatment of wounds in turtles are still anecdotal, and it is for this reason that it is important to report the experiences in native species. The objective of this report was to describe the resolution of dog bite trauma in 2 Chaco tortoises (*C. chilensis*) using 20 % propolis tincture.

Description of clinical cases

Case 1. An adult female Chaco tortoise, *C. chilensis*, weighing 660 g and body condition 3/5, was presented to the clinic. According to the tutor, the patient was attacked by a domestic canine at least 12 hours before the consultation. Clinical inspection confirmed the fracture of the carapace in the caudal portion, involving the loss of bone tissue from the 7th marginal scute, on both sides, as well as a significant portion of the 4th costal scute on the left side. It was possible to observe the exposure of connective tissue (coelom) and musculature of the area (Figure 1), perceiving a good vitality in the tissue.

The exposed bone tissue of the carapace was poorly developed, suggestive of metabolic bone disease. The latter coincides with the fact that the patient was fed lettuce, tomato and cucumber for more than 10 years.

Figure 1 Injury showing loss of bone tissue and exposure of connective tissue and muscle

Chemical restraint was performed, using midazolam at 1.5 mg/kg intramuscularly (IM)[11], due to the patient being enclosed in the shell. Local anesthesia was performed with 1 % lidocaine, topical and local infiltration. Once the animal was relaxed, an intraosseous access was placed in the gular shield with a 21 G needle, after antisepsis with 98 % alcohol, proceeding to hydration with lactated Ringer's at a rate of 20 mL/kg/day, for 48 h. The lesions were thoroughly cleaned with 0.5 % chlorhexidine + 4 % glycerin (Clorhpet® P'ludos®, Iris SAIC) and saline solution, removing surface contaminants with a soft brush. 3 hooks were fixed to the shell with epoxy resin, looking for intermediate points where to fix the skin. Caudal to each hook, approximation sutures were made with 0.30 mm polyamide, between the skin and the hooks, trying to avoid excessive tension between them (Figure 2).

Cefotaxime antibiotic treatment was established at a dose of 40 mg/kg, IM, every 24 h for 10 days[11] accompanied by tramadol 8 mg/kg, IM, every 48 h, for 10 days[11,12]. The open spaces between skin and shell were treated every 24 h, with 0.5 % chlorhexidine + 4 % glycerin (Clorhpet® P'ludos®, Iris SAIC), for 15 days, and covered with 20 % propolis tincture (isopropyl extract) every 48 h for 20 days.

The patient was kept in a reduced environment, on newspaper, during the entire evaluation period (5 weeks). The atmospheric variables were not manipulated for the treatment of the animal, registering during the evaluation period an average temperature of 27° C during the day and 15° C at night.

Forced feeding was carried out using a 12 G rigid gavage tube, performed on the basis of the maintenance metabolic rate (MMR) in tortoises, using the following formula: $MMR = K \times P^{0.75}$, where K is the constant (32 in turtles) and P the weight of the animal[4]. Thus, 24 kcal/day were administered, every 48 h, 2 times.

After 72 h posterior to the procedure, the intraosseous catheter was removed. The patient was placed in water baths for 60 min, every 72 h, drinking water normally from the first day, and the first voluntary intake could be verified after 5 days. Subsequently, the patient regularly ate cactus (*Opuntia* sp.), boiled squash (*Cucurbita* sp.) and commercial food for tortoises.

Figure 2 Use of hooks fixed to the carapace to perform approximation suture

Case 2. A specimen of the Chaco tortoise, *C. chilensis*, male, adult, weighing 520 g, body condition 2/5, was presented to the clinic. A level of dehydration of approximately 8 % was perceived, suggestive due to the sunken eyes. According to the tutor, the patient was attacked by a domestic canine of the Dogo Argentino breed, approximately 1 h before the consultation.

Clinical inspection confirmed the fracture of the carapace in the caudal portion, involving the loss of the 10th and 11th marginal scutes on the right side, a portion of the 9th marginal scute on the left side, the loss of the anal scute of the carapace, and the detachment of the 11th marginal shield on the left side, which remained adhered to the musculature. It was possible to observe the exposure of the muscles in the area (Figure 3), but perceiving a good vitality in the tissue. Plastron inspection revealed loss of bone continuity at the junction between the humeral and pectoral scutes, fracture and subsidence of the right femoral scute, loss of the right anal scute, and loss of bone continuity between the femoral and anal scutes on the left side (Figure 4).

Figure 3 Injury with loss of bone tissue and exposure of muscle tissue

Figure 4 Lesions with loss of continuity and bone tissue

Chemical restraint was performed using ketamine at a dose of 30 mg/kg and midazolam at a dose of 1.5 mg/kg, IM[11], due to the patient being enclosed in the shell. Tramadol at a dose of 10 mg/kg and meloxicam at a dose of 0.1 mg/kg, IM[11], were used as analgesics. After relaxation, after 30 min, vascular access was

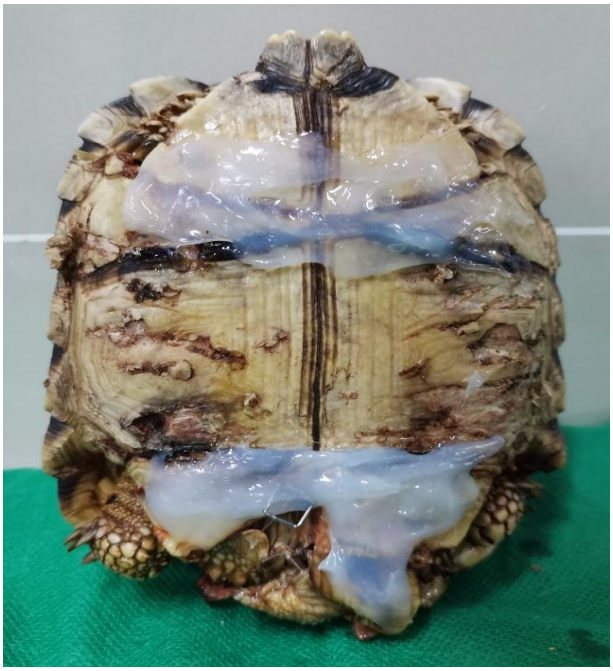
ensured in the right jugular vein with a 24 G catheter, and fluid therapy with lactated Ringer's was started. A K30 gastroesophageal tube was placed through an esophagostomy[4,13] for food administration. Local anesthesia was applied to the soft tissue lesions, with topical 1 % lidocaine and local infiltration. The lesions were thoroughly cleaned with 0.5 % chlorhexidine + 4 % glycerin (Clorhpet® P'ludos®, Iris SAIC) and saline solution, removing surface contaminants with a soft brush.

Figure 5 Use of epoxy resin and hooks fixed to the carapace to perform approximation suture

In the caudal carapace, the detached marginal scute was approximated and 3 hooks were fixed to the carapace with epoxy resin. Caudal to each hook, approximation sutures were made with 0.30 mm polyamide, between the skin and the hooks, trying to cover the muscle tissue with skin flaps (Figure 5).

In the plastron, the fractured bone fragments were approximated, immobilizing them with epoxy resin. A hook was fixed in the epoxy resin placed on the femoral shields. Caudal to the hook, 2 approximation sutures were made with 0.30 mm polyamide, between the skin and the hook, trying to cover the muscle tissue, reducing the dead space (Figure 6).

Figure 6 Approximation and immobilization of bone fragments, and approximation suture on exposed muscle tissue



Cefotaxime antibiotic treatment was established at a dose of 40 mg/kg, IM, every 24 h, for 10 days[11] accompanied by meloxicam at 0.1 mg/kg[11], IM, every 24 h, for 4 days, and tramadol at 10 mg/kg, IM, every 48 h, for 10 days[11,12]. The open spaces between skin and shell/plastron were treated every 24 h, with 0.5 % chlorhexidine + 4 % glycerin (Clorhpet® P'ludos®, Iris SAIC), for 10 days, and covered with 20 % tincture of propolis (isopropyl extract) every 48 h, for 30 days.

Fluid therapy was administered at a rate of 20 mL/kg/day as maintenance, added to 14 mL/day as deficit replacement, for 72 h, after which the intravenous catheter was withdrawn. The patient was placed in water baths for 30 min, being very uncomfortable in the water, so it was decided to do it every 5 days.

Forced feeding was carried out using the gastroesophageal tube, on the basis of the MMR in tortoises, using the following formula: MMR = K x $P^{0.75}$, where K is the constant (32 in turtles) and P is the weight of the animal[4]. Thus, 20 kcal/day were administered, every 24 h, for 17 days, accompanied by mineral water. The first voluntary intake could be verified on day 18, subsequently feeding regularly on cactus (*Opuntia* sp.) and papaya (*Carica papaya*).

Results

In case 1, after 15 days of evaluation, good adherence of the skin to the subcutaneous tissue was perceived, although there was hypersensitivity to manipulation of the affected area. After 5 weeks, the sutures were removed, the adhesion of the integument and stability of the wound were verified (Figure 7). At the subsequent evaluation, the removal of sutures, the patient did not present signs compatible with pain[14], keeping a normal behaviour. At no time during the evaluation period there were sero-hemorrhagic or purulent exudates, changes in the color of the surrounding skin, or the presence of odors indicative of necrosis.

In case 2, the sutures were removed at 5 weeks, the adhesion of the integument and stability of the wounds were verified (Figures 8 and 9). On evaluation after suture removal, the patient had a limp on the right side, which could be associated with pain[14]. At no time during the evaluation period there were sero-hemorrhagic or purulent exudates, changes in the color of the surrounding skin, or the presence of odors indicative of necrosis.

Figure 7 Integument in the process of resolution after the removal of the sutures

Figure 8 Integument in the process of resolution after the removal of the sutures

Figure 9 Integument in the process of resolution after the removal of the sutures

Discussion

The resolution time of wounds in turtles varies depending on their nutritional status, environmental temperature, the presence of foreign bodies or fluids in the wound, and infection[15], which is why it was important to provide nutritional monitoring to patients, in addition to drug treatment, and try to keep the animals within an optimal temperature range for their species, called POTZ (preferred optimal temperature zone). Although, in general, a healing time of between 6 and 8 weeks is mentioned before removing the sutures[16,17], the wounds in the 2 treated patients evolved rapidly, achieving good skin adhesion at 5 weeks. Wounds in turtles form a dry, persistent scab on the wound bed, and healing is characterized by epithelialization under the scab with decreased thickness of the dermis that slowly recovers, prolonging the inflammation phase until 28 to 42 days, while the proliferation of the connective tissue and its remodeling does not occur clearly before 42 to 135 days, and it is considered healed only when the scab is shed and the skin that remains exposed is completely epithelialized[16].

The reduction in time, compared to reports from other countries, could be due to the fact that propolis accelerates the tissue repair process due to the proliferation of fibroblasts and acceleration of the transformation from fibrocytes to fibroblast, favoring the synthesis and deposition of collagen fibers[18]. Propolis also increases the expression of several genes that promote wound healing, such as fibroblast growth factor 18 (FGF-18) and vascular endothelial growth factor A (VEGF-A)[10]. Successful healing cases have been described using propolis at different concentrations in species such as goats[18], pigs[19], canines[20,21], rabbits[22], guinea pigs[23], rats[24], and even humans[25]. Most of the in vivo studies on different wounds suggest beneficial effects on healing, however, there is still little information on the dose and side effects of propolis on wounds[9].

Honey has also been widely used in the management of skin wounds in wild animals, particularly turtles and birds[15], either because it promotes the healing process, as well as for its antibacterial properties[15,17]. A synergistic effect of bee honey with propolis has also been reported in a study on the healing of skin wounds in rats[26].

In cases such as those described, the use of 1% silver sulfadiazine could be considered, due to its ability to penetrate necrotic tissues and stimulate epithelialization, as well as hydrogels and hydrocolloids, which

help maintain wound moisture and facilitate autolytic debridement[15]. A study of wound healing by secondary intention in turtles reports that topical administration of insulin improves wound healing, shortening the time required for complete epithelialization[27].

For the repair of lesions, such as those reported in this paper, the administration of anesthetics and analgesics intrathecally[28] is suggested, but the technique requires a higher level of experience. Oral administration of tramadol could also be considered, which has reported good levels of analgesia in turtles[29], taking advantage of the use of tubes for force-feeding.

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Conflicts of interest

There is no conflict of interest, including financial, personal or other relationships with other persons or organisations that could inappropriately influence the work

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Ethical considerations

During the clinical cases we did not incur in professional misconduct, we have taken into account human sensitivity towards living beings without discomfort, anguish and adequate pain management, for this we took into account the use of adequate analgesia protocol and a patient follow-up plan.

Contribution of the authors in the article

The authors were involved in the planning of surgical procedures and systemic and topical treatments, as well as in drafting and revising the manuscript.

Research limitations

The limitation in the present report was that a visual and clinical evaluation of wound evolution was performed. For a correct evaluation of the effects of propolis on the wound healing process in tortoises, an experimental study should be carried out, evaluating the evolution of the wounds by means of histo-logical techniques in experimental and control groups, duly accredited by a bioethics committee. The present work reports 2 cases of patients who came to the clinic and not a planned methodological study with antelation.

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