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CASE REPORT
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Primary Perianal Malignant Melanoma in a Dog - Combination Therapy

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ABSTRACT

***Background*:** Melanocytic neoplasm can arise from melanocytes in any location of the body. Malignant melanoma (MM) has a poor prognosis in dogs and presence of lymphvascular invasion, distant metastasis, or mitotic activity present prognostic value. Primary melanoma affecting the gastrointestinal tract has been rarely reported in veterinary literature, thus the prognosis affecting gastrointestinal tract is unknown. Electrochemotherapy (ECT) is an effective local treatment which combines chemotherapeutic drugs mainly bleomycin or cisplatin followed by the delivery of permeabilizing electrical pulses However, other hydrophilic drugs seem to present an increase cytotoxic effect such as carboplatin.

***Case*:** A 9-year-old mixed-breed neutered dog was referred to a private clinic with a mass in the perianal region diagnosed as perianal melanoma. No metastasis was observed on abdominal ultrasound nor chest x-ray (3 views). Clinical signs noted were tenesmus, hemorrhagic discharge, weight loss and hyporexia. Considering the tumor volume (16.0 x 10.0 cm), a neoadjuvant ECT session was proposed. The authors opted for carboplatin (300 mg/m^2, intravenously), administered over 20 min and cisplatin intratumorally (1 mg/cm^3, equivalent to 1 mL/1cm^3 total volume 20 mL) administered in the upper parts of the mass that could be reached while avoiding drug leakage. After administration, sequences of eight biphasic pulses, (100 microseconds), with a voltage ranging from 650-1,000V/cm (pulse generator Onkodisruptor®) using a hexagonal/single pair and plate electrode were delivered. At day 30th, a partial response was observed accordingly to RECIST system, with tumor size of 5.0 x 5.0 cm (65.4 cm^3). A second ECT session was performed with the same previous protocol, but with a decreased dosage of carboplatin (240 mg/m^2 consistent with 20% reduction) due to adverse effects in the first session, resulting in stable disease at day 60th (30 days after second ECT). Then, we proposed a surgical excision of the mass including partial resection of ventral rectum with intraoperative ECT. Afterwards, it was observed fecal incontinence that did not resolved after time but did not significant cause a morbidity in the patient. Patient achieved a disease-free interval (DFI) of 700 days and survival time of 730 days. Unfortunately, patient died due to distant metastasis.

***Discussion*:** Surgery is still the cornerstone treatment for MM in dogs, regardless anatomic site. However, in perianal region, wide or radical local surgical excision is a challenge due to anatomic region which precluded most of the time to achieve complete margins. In this report, the origin of the tumors was not defined since no normal tissue was found surrounding tumors cells probably due to tumor invasion and destruction of surrounding tissue. Thus, based on the previous literature, this tumor could have been arisen from rectum wall or anal sac. The longer DFI and survival time from this patient is superior from the most veterinary cases in literature which combined different types of treatment such as surgery, chemotherapy, immunotherapy, palliative care or ECT. Neoadjuvant ECT leaded to a reliable approach for partial remission in order to perform a better surgical approach in this case report. To conclude, ECT may be an option for partial remission and local control in regions which anatomic limitation is a challenge for wide excision.

Keywords: bleomycin, carboplatin, cisplatin, electroporation, melanocytic tumor.

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INTRODUCTION

Melanocytic tumors are common in dogs and accounts up to 7% of all malignant tumors with oral cavity. Can arise from melanocytes in any location with oral cavity the most affected site, followed by lips, skin and digit [3]. Oral malignant melanoma (MM) has a poor prognosis in dogs and presence of vascular invasion, distant metastasis, nuclear atypia, and mitotic activity present prognostic value [6]. Primary melanoma affecting the gastrointestinal tract has been rarely reported in veterinary literature and the previous described sites were colon, and rectum, [1,8]. Other sites such as perianal and anal sac are also rarely reported [4,11]. Due the lack of reports in these sites, the prognosis of MM affecting gastrointestinal tract is unknown.

Electrochemotherapy (ECT) is an effective local treatment which combines the administration of chemotherapeutic drugs mainly bleomycin or cisplatin, followed by the delivery of permeabilizing electrical pulses [9]. However, other hydrophobic drugs seem to present an increase cytotoxic effect such as carboplatin. In veterinary medicine, ECT has been widely used due to its high efficacy in all solid tumors, such as cutaneous and subcutaneous tumors, skin metastasis, melanoma, sarcomas, and visceral tumors [2,9].

This report aimed to describe a canine primary perianal malignant melanoma treated with neoadjuvant ECT protocol (intravenous carboplatin plus intratumorally cisplatin), and a sphincter sparing surgery associated with intraoperative ECT with long-lasting complete response.

CASE

A 9-year-old mixed-breed neutered dog was referred to a private clinic with a mass in the perianal region without anamnesis history. In the records, antibiotics and non-steroidal anti-inflammatory were prescribed for 14 days without tumor remission. A fine-needle-aspiration was performed confirming a melanoma (tumor size of 3.0 x 2.0 cm). No metastasis was observed on abdominal ultrasound nor chest x-ray (3 views), and staging was based on World Health Organization. The dog underwent two sessions of cryosurgery with 21 days interval without success (progressive disease was observed; tumor size of 16.0 x 10.0 cm). Clinical signs noted were tenesmus, hemorrhagic discharge, weight loss and hyporexia.

Considering the tumor volume, a neoadjuvant ECT session was proposed. The authors opted for carboplatin[1] [300 mg/m^2, i.v. - administered over 20 min] and cisplatin[1] [intratumorally - 1 mg/cm^3, equivalent to 1 mL/1cm^3; total volume 20 mL] administered in the upper parts of the mass that could be reached while avoiding drug leakage. The protocol of carboplatin plus intratumorally cisplatin was indicated due to lack of commercial bleomycin in that moment, and due to carboplatin be the first line treatment of melanoma. After the administration, sequences of eight biphasic pulses, (100 microseconds duration), with a voltage ranging from 650-1,000V/cm (pulse generator Onkodisruptor®[2]) using a hexagonal/single pair and plate electrode were delivered. The procedure was performed under general anesthesia. An intense leukopenia post-ECT with carboplatin plus cisplatin was observed (total leukocytes 1,000 cells/mm^3) at day 7th with hyporexia and fever. Supportive care was administered with antibiotics and fluid therapy resulting in patient's recovery within 7 days. At day 30th, a partial response was observed accordingly to RECIST system, with tumor size of 5.0 x 5.0 cm (65.4 cm^3). A second ECT session was performed with the same previous protocol (carboplatin plus intratumorally cisplatin), but with a decreased dosage of carboplatin (240 mg/m^2 consistent with 20% reduction) due to adverse effects in the first session, resulting in stable disease at day 60th (30 days after second ECT) [Figure 1]. Then, we proposed a surgical excision of the mass including partial resection of ventral rectum with intraoperative ECT [rescue protocol using solely bleomycin intravenous at a dosage of 15,000 UI/m^2]. This approach was opted due to limitation response after second ECT and a rescue protocol was used because patient did not show clinical response with carboplatin plus cisplatin combined with electric pulses after two sessions of ECT in a 30-days interval. In this moment we imported a commercial bleomycin[3] for use in the rescue protocol.

Before surgery, we opted to pass a urethral catheter number 10 and number 8 through rectum and urethra, respectively, for better visualization of the structures. A 2-cm surgical incision was made through the skin and soft tissue adjacent to the tumor tissue. A Metzenbaum scissor was used to dissect peritumoral tumor adjacent to tissue. It was observed that the MM was passing ventrally to the distal rectum without invasion of the structures. The adherence in the distal

rectum was release gently with minimal trauma. Around 2/3 of the distal rectum was resected in its lateral and ventral region, sparing the dorsal part of the external anal sphincter and dorsal rectum. After removal of MM, intraoperative ECT was performed among all the area and on surface of the rectum with the rescue protocol previous cited. Afterwards, subcutaneous tissue was closed using Cushing pattern (Nylon 2-0)[4], and dermorraphy was made using appositional suture bringing the tissue in direct approximation of the 2 cutting surfaces in a simple interrupted pattern (Figure 2).

The mass was sent for histopathological evaluation and revealed a malignant round cell proliferation composed by cells with moderate anisokaryosis, multiple nucleoli and low cell pigmentation (Figure 3). The mitotic index was 13 and no surrounding normal tissue was identified in the histopathological analysis.

Post-surgery the patient was release with enrofloxacin[5] [5 mg/kg, b.i.d, PO, 7 days], metronidazole[6] [25 mg/kg, b.i.d, PO, 7 days], meloxicam[5] [0.1 mg/kg, s.i.d, PO, 5 days], dipyrone[7] [25 mg/kg,t.i.d, PO, 5 days] and canned food. Afterwards, it was observed fecal incontinence that did not resolved after time but did not significant cause a morbidity in the patient. During follow up patient achieved disease free interval (DFI) of 700 days and survival time of 730 days. Unfortunately, patient developed distant metastasis (pulmonary) and cutaneous metastasis confirmed by chest x-ray and cytology, and owner opted for euthanasia.

DISCUSSION

Surgery is still the cornerstone treatment for MM in dogs regardless anatomic site [6,11]. However, in perianal region, wide or radical local surgical excision is a challenge due to anatomic region which precluded most of the time to achieve complete margins. Furthermore, rectum wall, and external anal sphincter must be resected due to its invasiveness leading most of patient incontinence [4,11]. In this case, the origin of the tumors was not defined since no normal tissue was found surrounding tumors cells, probably due to tumor invasion and destruction of surrounding tissue. Thus, based on the previous literature, this tumor could have been arisen from rectum wall or anal sac.

In our patient, due to the advanced stage, we proposed a neoadjuvant ECT treatment in order to better surgically approach. ECT has been used to treat solid tumors that combine antineoplastic drugs with electric pulses [2,9]. A partial remission was observed 2 months later therapy to perform surgery. It is worth to be mentioned that this is the first *in vivo* report which combined i.v. carboplatin plus local cisplatin with electrochemotherapy in a neoadjuvant protocol. In order to preserve the external anal sphincter, a sparing sphincter surgery was performed in agreement with owners who would not like a radical surgery. It has been demonstrated *in vitro* that ECT with carboplatin using an equine sarcoid cell line increased 3-fold in the toxicity with an electric field of 1000V/cm. Furthermore, the same

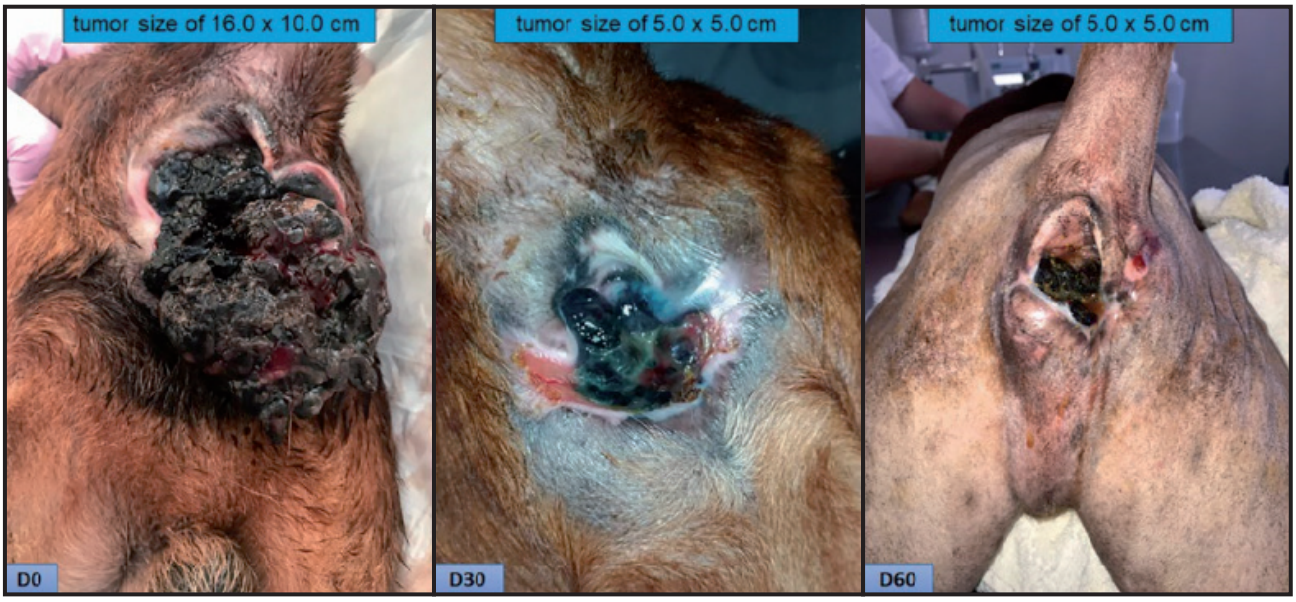

Figure 1. Primary perianal malignant melanoma (MM) in a 9-year-old mixed-adult dog. A- Perianal MM before ECT procedure measuring 16.0 x 10.0 cm. B- At day 30th, a partial response was observed with tumor size of 5.0x5.0 cm (65.4 cm^3). C- After 2 sessions of ECT, a partial remission was observed (at day 60^{th}) with tumor size of 5.0x5.0 cm (65.4 cm^3).

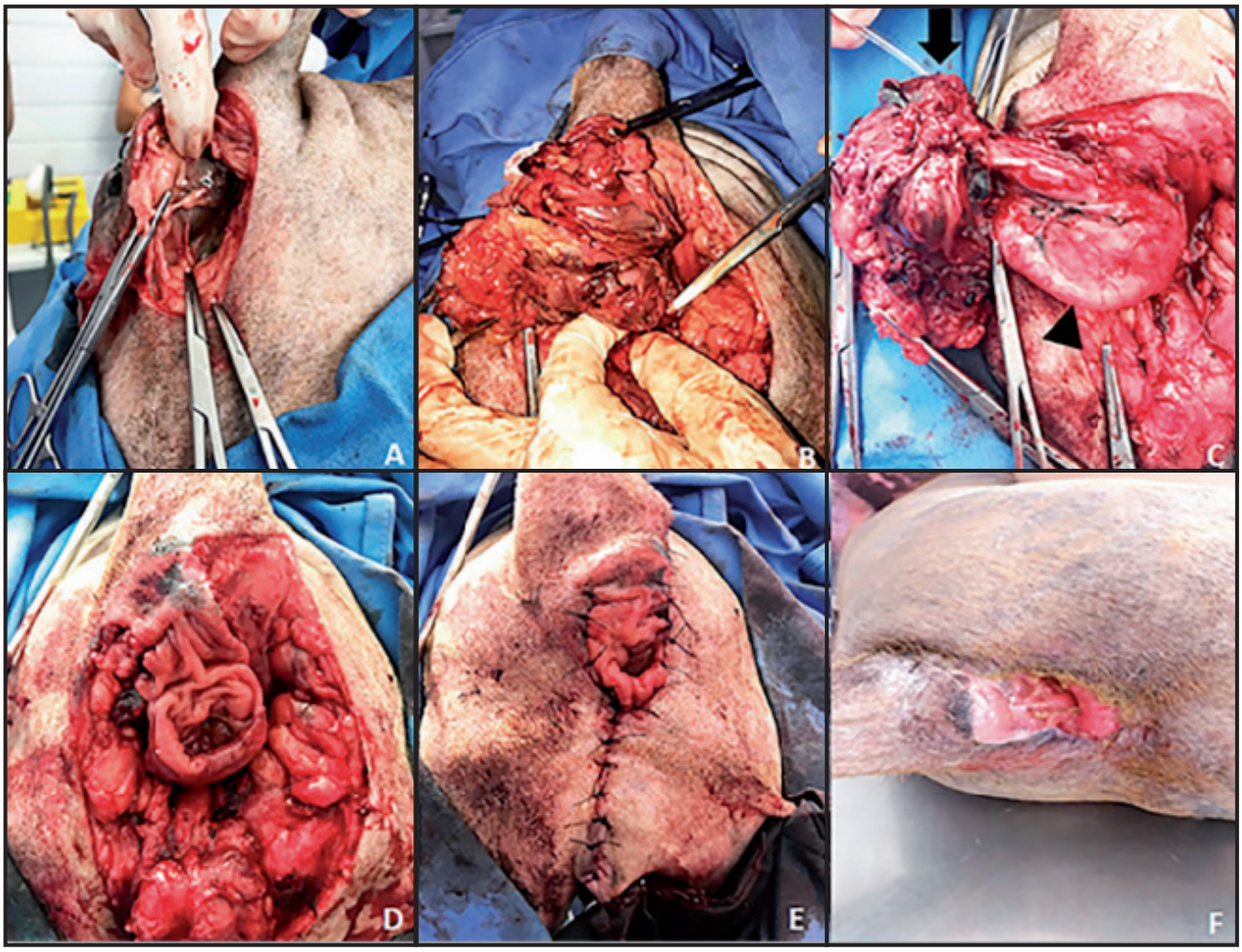

Figure 2. Primary perianal malignant melanoma in a 9-year-old mixed-breed neutered dog. A- It was made an elliptical incision lateral to the anus through the skin adjacent to the tumor. B- The subcutaneous tissues were incised, exposing the pelvic diaphragm. The fascia between the external anal sphincter and the levator ani muscle was separated, allowing visualization of the lateral aspect of the rectum, which was compromised by neoplastic tissue. C- The lateral incision was widened around the entire anus, allowing better rectal exposure. The rectal wall was everted through the anus with the placement of sutures. A full-thickness incision was made, resecting the neoplastic segment. D- The apposition of the edges was performed with simple interrupted sutures. The external anal sphincter and the levator ani muscles were replaced with interrupted appositional sutures. E- Subcutaneous tissues and skin were routinely closed. F- Skin wound healing 15 days after surgery.

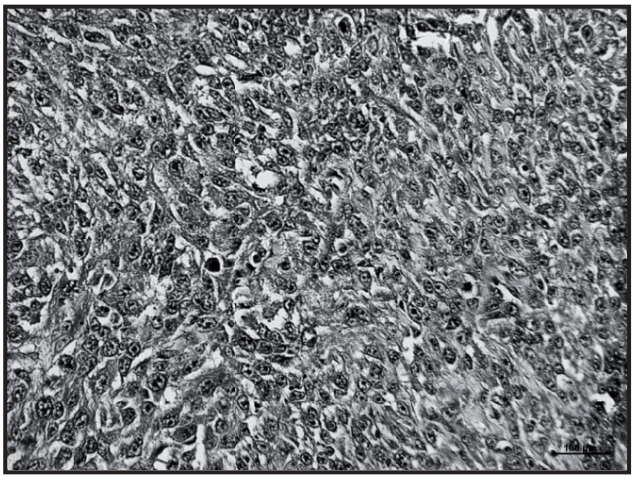

Figure 3. Histological analysis of a canine melanoma located in the perianal region. It was observed a proliferation of malignant cells with moderate anisokaryosis and multiple nucleoli. Cells shows low degree of pigmentation [HE; 40x].

study suggested that the dose of carboplatin could be reduced by 2.8 times when combined with irreversible electroporation compared to carboplatin alone [10].

The most common clinical signs of anorectal mass include anorectal bleeding, pain, and tenesmus as observed in this case and in literature [1,4,8,11]. During follow-up cutaneous and distant metastasis was observed. Ultrasound did not detect any abnormality in regional lymph nodes from the perianal region which included internal (hypogastric), medial and external iliac lymph nodes.

Other sites such as perianal and anal sac are also rarely reported [4,8,11]. The previous description reports overall survival rates between 0 up to 1740 days [4,8,11]. Based on previous reports, this region

appears to present an aggressive clinical behavior associated with poor survival times. We observed the same survival time (730 days) as reported in literature. In a retrospective study with 11 dogs diagnosed with malignant anal sac melanoma, regardless of treatment (surgery, chemotherapy, immunotherapy, or palliative), median survival time was short (107 days) [11].

Other report using electric pulses with antineoplastic drugs for local control achieved a partial remission for a canine anal melanoma that lasted 3 months before metastasized to sublumbar lymph nodes [8]. The DFI (700 d) and survival time (730 d) from this patient is superior from the most veterinary cases in literature which combined different types of treatment such as surgery, chemotherapy, immunotherapy, palliative or ECT ranged from 0-1740 days [1,4,8,11]. In human literature, a woman diagnosed with perineal MM was successfully treated with ECT presenting complete remission after 2 sessions with progression free survival of 2.5 years [5] suggesting that this therapy may be an effective alternative to conventional treatments.

Although the presence of vascular invasion, distant metastasis, nuclear atypia, and mitotic activity are associated with poor prognosis. In our case, we did not found presence of vascular nor lymph vessel invasion, a low cell pigmentation and mitotic index of 13 was observed and no surrounding normal tissue was identified in the histopathological analysis. The evaluated cells presented a moderated pleomorphism with infiltration of adjacent structures. Although the tumor had an infiltrative size, the treatment provided a good outcome. One of the theories that this patient presented long-lasting complete remission might be the dramatically remission from the first ECT session releasing tumor associated antigens boosting immunology system as observed in another case report [7]. Although we thought that the presence of severe leukopenia would be a limited factor response, this adverse effect did not interfere in the response treatment of this case report.

Primary perianal malignant melanoma has been rarely reported in veterinary literature with unknown prognosis. This is the first case to use systemic carboplatin in an electrochemotherapy protocol as neoadjuvant treatment. Neoadjuvant ECT leaded to a reliable approach for partial remission in order to perform a better surgical approach in this case report. ECT may be an option for partial remission and local control in regions which anatomic limitation is a challenge for wide excision.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of paper.

REFERENCES

1 **Clarke L.L. & Rissi D.R. 2018.** Malignant rectal melanoma in 2 dogs. *Canadian Veterinary Journal*. 59: 152-154.

2 **Dos Anjos D.S., Bueno C., Magalhaes L.F., Magalhaes G.M., Mattos Jr. E., Pinto M.M.R., De Nardi A.B., Brunner C.H.M., Leis-Filho A.F., Calazans S.G. & Fonseca-Alves C.E. 2019.** Electrochemotherapy induces tumor regression and decreases the proliferative index in canine cutaneous squamous cell carcinoma. *Scientific Reports*. 9: 15819.

3 **Goldschmidt M.H. & Shofer F.S. 1992.** Skin tumors. In: *Skin Tumors of the Dog and Cat*. Oxford: Butterworth Heinemann, pp.142-151.

4 **Kim D.Y., Mauldin G.E., Hosgood G. & Cho D.Y. 2005.** Perianal malignant melanoma in a dog. *Journal Veterinary Internal Medicine*. 19(4): 610-612.

5 **Kubota Y., Tomita Y., Tsukigi M., Kurachi H., Motoyama T. & Mir L.M. 2005.** A case of perineal malignant melanoma successfully treated with electrochemotherapy. *Melanoma Research*. 15(2): 133-134.

6 **Smedley R.C., Spangler W.L., Esplin D.G., Kitchell B.E., Bergman P.J., Ho H.Y., Bergin I.L. & Kiupel M. 2011.** Prognostic Markers for Canine Melanocytic Neoplasms: A Comparative Review of the Literature and Goals for Future Investigation. *Veterinary Pathology*. 48(1): 54-72.

7 **Spugnini E.P., Dragonetti E., Vincenzi B., Onori., Citro G. & Baldi A. 2006.** Pulse-mediated chemotherapy enhanceslocal control and survival in a spontaneous canine model of primarymucosal melanoma. *Melanoma Research*. 16: 23-27.

8 Spugnini E.P., Filipponi M., Romani L., Dotsinsky I., Mudrov N., Baroni A., Ruocco E., Laieta M.T., Montesarchio V., Cassandro R., Citro G. & Baldi A. 2007. Local control and distant metastasis after electrochemotherapy of a canine anal melanoma. *In vivo*. 21: 897-900.

9 Spugnini E.P. & Baldi A. 2019. Electrochemotherapy in Veterinary Oncology: State-of-the-Art and Perspectives. *Veterinary Clinics North American Small Animal Practice*. 49: 967-979.

10 Souza C., Villarino N.F., Farnsworth K. & Black M.E. 2017. Enhanced cytotoxicity of bleomycin, cisplatin, and carboplatin on equine sarcoid cells following electroporation-mediated delivery *in vitro*. *Journal Veterinary Pharmacology Therapeutics*. 40(1): 97-100.

11 Vinayak A., Frank C.B., Gardiner D.W., Thieman-Mankin K.M. & Worley D.R. 2017. Malignant anal sac melanoma in dogs: eleven cases (2000 to 2015). *Journal of Small Animal Practice*. 58: 231-237.