CLINICAL REPORT

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# Preliminary assessment of electrochemotherapy feasibility in dogs with vesical transitional cell carcinoma

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## Abstract

Electroporation is a technique that increases the uptake of chemotherapeutic drugs by tumors. Electrochemotherapy (ECT) has been successfully used to treat solid tumors. Recently, novel applications have been explored in the treatment of visceral tumors. This report aimed to describe the ECT as an approach to vesical carcinoma in three dogs. The patients received ECT with bleomycin as an intravenous bolus and intra-lesional cisplatin (cases 2 and 3). The ECT was performed by electroporator (Onkodisruptor®) using a plate and/or a single pair needle array electrode. Case 1 was a 7-year-old female Pitbull dog with a history of hematuria and stranguria. The ECT was performed during cystotomy using a single pair array electrode. However, the patient developed uroabdomen two days post-ECT and died 5 days later. Case 2 was a 12-year-old female Poodle dog with hematuria, dysuria, and pollakiuria. Cystotomy and ECT were performed using plate array electrodes. Complete remission of the intra-luminal mass was observed 11 days post-ECT. However, 21 days after the procedure, an acute unilateral renal failure occurred possibly due to a neoplastic embolus into the right ureter leading to kidney hydronephrosis, and the patient was euthanized. Case 3 was a 10-year-old female Cocker dog with hematuria and pollakiuria. The patient was fully competent after ECT without clinical signs of pollakiuria and recovered from hematuria 7 days post-ECT. The bladder returned to normal status 28 days post-ECT. The ECT was not able to increase the overall survival of the patients evaluated and should be indicated carefully.



## Introduction

Electrochemotherapy (ECT) is a non-thermal ablative technique that combines high-intensity electric pulses (electroporation) with antineoplastic drugs, enhancing their effects by increasing cellular uptake.[1] In human and veterinary medicine, ECT has been widely used due to its high efficacy in solid cutaneous and subcutaneous tumors and more recently in visceral tumors including thymoma, liver and pancreatic carcinoma.[1-7]

*In vivo* studies conducted in mice with implanted human bladder cancer supported the effectiveness of ECT in this tissue since reported response rate after ECT was 100% and 47.00% using mitomycin C and cisplatin, respectively.[8] In dogs, urinary bladder tumors account for about 2.00% of all canine tumors, being transitional cell carcinoma (TCC) the most common histological subtype.[9] In the bladder, TCC locates typically in the trigone region and commonly involves the urethra or prostate.[9,10] One of the main challenges for TCC is the local control since complete excision is often unfeasible in dogs with urethral, prostatic, or ureteral involvement.

Usually, systemic chemotherapy (i.e., mitoxantrone, vinblastine, and carboplatin) is the standard-of-care

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treatment for TCC in combination with non-steroidal anti-inflammatory drugs (NSAIDs) with median survival times of 6 to 12 months. Surgery, when possible, and other options including radiation therapy, laser ablation, and stenting may improve survival.[9]

This case report aimed to describe the clinical outcomes from three cases of canine TCC treated with ECT using bleomycin or bleomycin in combination with intratumorally cisplatin.

## Case Description

All animals included in this study were client-owned and informed consent was obtained before any treatment. All dogs presented T2 staging (tumor invading the bladder wall) and owners declined partial or total cystectomy and/or conventional chemotherapy treatment schedule (as first-line treatment). We followed recommendations for reporting clinical studies on ECT.[11] All patients received ECT under general anesthesia and bleomycin (Bleocris®; Gador, Buenos Aires, Argentina) at a dosage of 15 unit per $m^2$ intravenously (IV). Five min after administration, five trains of eight biphasic pulses at the voltage of 800 V per cm, 1.00 Hz frequency, lasting 50 + 50 µsec with 300 µsec interpulse (total treatment time per train 3.20 ms) were administered using a clinical electroporator certified for veterinary application (Onkodisruptor®; Biopulse Ltd., Naples, Italy) using a plate and/or a single pair needle array electrode until complete coverage of the lesion. Cases 2 and 3 received intra-lesional cisplatin (C-platin®; Blau Laboratory, São Paulo, Brazil) at a dose of 1.00 mg per $cm^3$ in addition to bleomycin. The bladder tumor volume was calculated using the following formula:

$$V\,(cm^3) = ab^2\,\pi/6$$

where, *a* is the larger diameter of the tumor nodule and *b* is the diameter of the tumor nodule perpendicular to *a*, as previously described.[12] For response evaluation, the reported criteria in solid tumors were used.[13]

**Case 1.** A 7-year-old female Pitbull dog was presented with a history of hematuria and stranguria. On physical examination, the patient presented abdominal distention and a palpable mass on bladder topography. Abdominal ultrasound showed a thick and irregular bladder wall and a mass (unmeasurable) in the trigone region. A biopsy sample was collected through cystoscopy. The thoracic radiograph revealed normal lungs. The histopathology reported a non-papillary infiltrative TCC. According to the World Health Organization staging system[14], the patient has corresponded to the histological staging method of T2N0M0. The owner declined conventional chemotherapy and opted for ECT. The ECT was performed using a single pair array electrode after tumor visualization by cystotomy. The animal developed uroabdomen two days post-ECT; characterized by abdominal distention and urine leakage from the bladder wall. Emergency care was instituted and the patient was referred to surgery, where severe hemorrhagic fluid was drained from the abdominal cavity. The bladder presented a dark region on the wall suggestive of necrosis (Fig. 1), which was surgically removed, but the patient died 5 days later. Necropsy was not authorized by the owner.

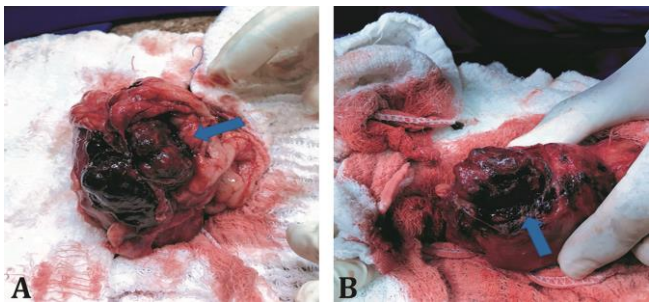


**Fig. 1.** A 7-year-old female Pitbull dog diagnosed with transitional cell carcinoma. **A)** Note the adherence of epiploon around the external wall of the bladder (arrow) with a hemorrhagic-dark lesion on it. **B)** Partial rupture with urine leakage (arrow).

**Case 2.** A 12-year-old female Poodle dog was presented with a non-papillary infiltrative TCC in the trigone region of the bladder treated previously for two months with vinblastine at every 14 days (2.50 mg per $m^2$; IV) and piroxicam (0.30 mg $kg^{-1}$ once daily, orally) until progressive disease. Clinical signs involved hematuria, dysuria, and pollakiuria. The ECT treatment was suggested. On abdominal ultrasound, the tumor was measured 2.00 × 1.76 cm, and a thick bladder wall in the trigone region with no hydronephrosis was observed (Fig. 2A). The histological staging method has corresponded to T2N0M0. The ECT was performed using plate array electrodes (Fig. 2B). Tumor volume was 3.24 $cm^3$ corresponding to 3.20 mL of intra-lesional cisplatin. Case 2 showed complete remission 11 days post-ECT on ultrasound (Fig. 2C), however, a sacculation was observed lateral to the vesical bladder in addition to an embolus (hyperechogenic mass without an acoustic shadow of 0.34 cm) inside right ureter leading to kidney hydronephrosis with dilatation of renal pelvis (1.50 cm). Because of high suspicion of vesical fistulae, a new exploratory surgery was performed confirming that sacculation was the dilatation of right ureter without evidence of primary tumor exhibiting only necrotic tissue (Fig. 2D). The embolus was released manually through gentle squeezing of the right ureter towards the vesical lumen confirming neoplastic necrotic embolus. The patient was fully competent after the procedure without clinical signs of hematuria. However, 21 days after the procedure the patient developed acute renal failure and pancreatitis. The acute unilateral renal failure was suspected to be a consequence of a neoplastic embolus as previously visualized on the ultrasound exam. Unfortunately, the owner chose euthanasia and necropsy was not performed.

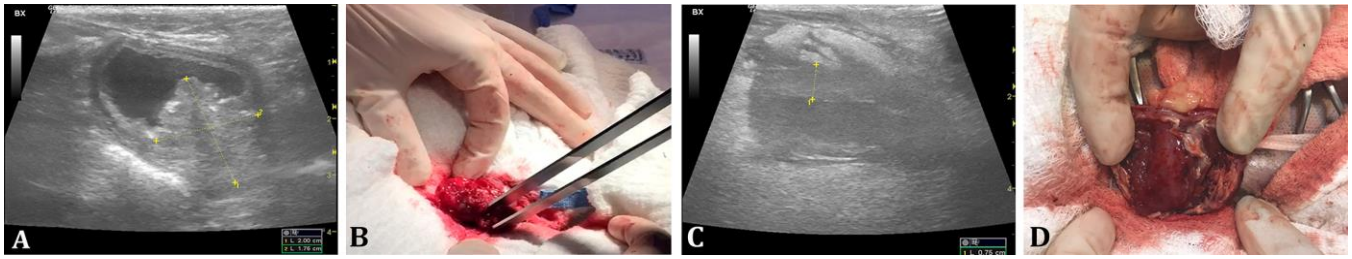


**Fig. 2.** A 7-year-old female Poodle dog diagnosed with transitional cell carcinoma (TCC). **A)** Ultrasound image showing TCC in the trigone region. **B)** Intra-operative electrochemotherapy (ECT) procedure applying 800 V per cm electric pulses on the bladder mass. **C)** 11 days post-ECT showing complete remission of intra-luminal mass, but the thickness of the bladder wall can be observed (calipers). **D)** Macroscopic aspect of the bladder with necrotic tissue having no macroscopic evidence of tumor.

**Case 3.** A 10-year-old female Cocker dog was referred with non-papillary infiltrative TCC in the trigone region (3.26 × 1.60 cm) with evidence of neoplastic emboli in lymphatic vessels. No hydronephrosis was detected. Clinical signs included hematuria and pollakiuria. The histological staging method has corresponded to T2N0M0. The oral firocoxib was prescribed (5.00 mg $kg^{-1}$, SID; Merial, São Paulo, Brazil). However, disease progression occurred one week afterward, and the owner elected ECT. Tumor volume was 4.00 $cm^3$ corresponding to 4.00 mL of intra-lesional cisplatin. The ECT was performed using a plate and a single pair array electrode (Fig. 3). The patient was fully competent after ECT without clinical signs of pollakiuria and recovered from hematuria seven days post-ECT. Meloxicam (0.10 mg $kg^{-1}$, once daily; Ourofino, Cravinhos, Brazil) administration was maintained after the procedure for seven days. On the ultrasound examination after seven days, a thick bladder wall due to inflammation or tumor infiltration was visualized. Twelve days after ECT, abdominal discomfort and urine incontinence were observed on physical examination. Oral oxybutynin (0.20 mg $kg^{-1}$, BID, five days; Apesen Laboratory, São Paulo, Brazil) was pre-scribed and the dog showed improvement of clinical signs. In this case, urine incontinence can be a result of the overactive bladder after ECT procedure due to intense inflammatory process and edema that may lead to the bladder wall smooth muscle involuntary spasms, which resolved with an antimuscarinic drug. A new ultrasound was performed 20 days after the procedure and showed no evidence of intra-luminal mass and a lower bladder wall measuring 0.60 cm (1.60 cm in the previous exam). The bladder returned to normal status 28 days after the ECT procedure (Fig. 4).

Forty days after ECT, the patient presented circling, head pressing, seizures, and epistaxis. The patient was suspected of cerebral metastasis due to evidence of neoplastic emboli in lymphatic vessels on previous histopathological results. Unfortunately, the patient died and partial necropsy was allowed only in abdominal and thoracic cavities. No macroscopic lesion was observed in any organ (Fig. 5), confirming a complete remission after ECT treatment in this case.

## Discussion

Survival time of cases 1, 2, and 3 were corresponded to 5, 30, and 40 days, respectively. The effectiveness of ECT with bleomycin and/or intra-lesional cisplatin in bladder cancer may promote local control as observed in cases 2 and 3. Limitations of these case series included the lack of a control group to compare response rates and survival times with mainstay treatments and the absence of histopathological biopsies after the procedure. In case 1, it was believed that the bladder necrosis may have occurred because of an extensive neoplastic lesion (14.00 ×13.00 cm approximately). A large tumor burden in the bladder wall may be a risk factor for bladder wall necrosis after ECT. The fact that the tumor infiltrates the muscular layer and produces wall induration (stage 2) indicates that after cell death, cellular cohesion can be lost, resulting in leakage of urine to the abdomen.

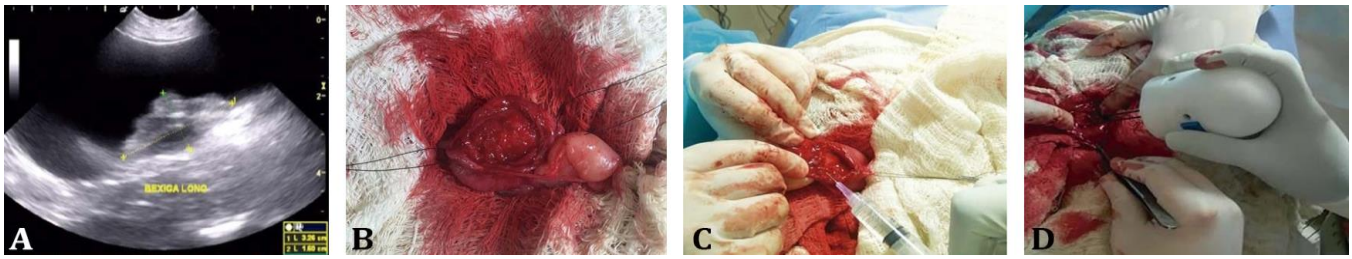


**Fig. 3.** A 10-year-old female Cocker dog diagnosed with non-papillary infiltrative transitional cell carcinoma in the trigone region. **A)** Ultrasound exam revealed a mass with mixed echogenicity in the bladder. **B)** The total exposure of the bladder through cystotomy to perform a correct application of electrochemotherapy (ECT) electric pulses. **C)** Intra-operative application of intra-lesional cisplatin (1.00 mg per $cm^3$). **D)** The ECT procedure with a single pair electrode applying 800 V per cm of electric pulses.

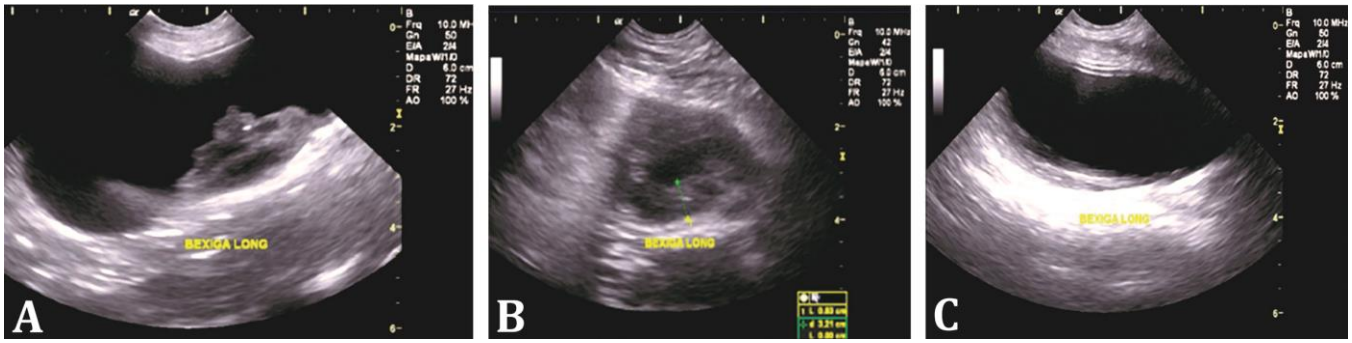


**Fig. 4.** Longitudinal and transversal ultrasound of the bladder in a Cocker diagnosed with transitional cell carcinoma (case 3). **A)** Tumor before the procedure. **B)** Seven days after the procedure, the thickness of the bladder wall suggestive of edema and inflammatory process. **C)** 28 days after electrochemotherapy procedure with complete remission of the tumor.

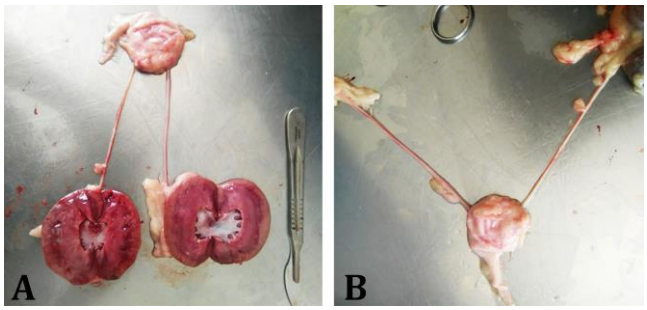


**Fig. 5.** Urinary system necropsy of case 3. No macroscopic alterations regarding transitional cell carcinoma were observed in **A)** kidneys, and **B)** internal or external bladder and ureter.

In case 2, complete macroscopic remission after ECT was confirmed, suggesting that ECT may be beneficial regarding local tumor control, as seen in other carcinomas. A neoplastic embolus is a risk factor of organ dysfunction as seen in this case, however, tumor invasion or a *de novo* lesion in the right ureter cannot be ruled out. Lesions involving ureters can be difficult to treat by ECT; therefore, ureter involvement evaluation is suggested before any ECT attempt.

According to Vásquez *et al*.,[8] ECT could be used immediately after transurethral bladder tumor resection or if recurrence is observed. Another interesting use of ECT is in cases of hemorrhagic tumors to stop bleeding.[15] New electrodes adaptable to cystoscopes are in demand for a better approach to bladder cancer to avoid open surgeries, diminishing anesthesia volume, and recovery time.

In conclusion, it can be suggested that ECT may exert local control of TCC in bladder resulting in clinical responses. Dogs being treated with ECT may also need systemic chemotherapy as metastatic diseases continue to be a concern in TCC. Taking into consideration the possibility of partial bladder wall rupture and hydronephrosis, careful patient choice and frequent monitoring are needed for this procedure as observed in these case series. The main key clinical message from this research is to warn practitioners that careful management using ECT is needed since no prospective study has been published about its use in canine bladder tumors. Further investigations are warranted to standardize ECT for bladder TCC and to identify patients that will benefit from this approach.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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