in vivo *26*: 931-936 (2012)

Palliative Intralesional Interleukin-2 Treatment in Dogs with Urinary Bladder and Urethral Carcinomas

URSULA KONIETSCHKE[1], ERIK TESKE[2], KONRAD JURINA[1] and CHRISTIAN STOCKHAUS[1]

[1]*Small Animal Hospital, Haar, Germany;*
[2]*Department of Clinical Sciences of Companion Animals, Utrecht University, Utrecht, The Netherlands*

Abstract. *Aim: The investigation of the influence of intralesional interleukin-2 (IL-2) on the clinical course and tumor progression in dogs suffering from urinary bladder and urethral carcinomas. Materials and Methods: Medical records of 25 dogs diagnosed with advanced transitional cell carcinomas (TCC) were retrospectively reviewed. In 14 dogs, intralesional IL-2 treatment was performed by transabdominal ultrasound-guided injection. Seven dogs underwent cytoreductive surgery, followed by IL-2 injection into the tumor bed. All dogs received long-term non-steroidal anti-inflammatory drugs. Results: Adverse effects associated with IL-2 treatment were not observed. At re-examination, 17 dogs showed marked clinical improvement and regression of tumor size. Four dogs were in complete remission. Conclusion: Intralesional IL-2 application is a safe and minimally-invasive palliative treatment option in dogs suffering from advanced transitional cell carcinoma when surgical cure is impossible. Prognosis depends on tumor localization and feasibility of concomitant cytoreductive surgery.*

Invasive transitional cell carcinoma (TCC) is the most common form of urinary bladder cancer in dogs and frequently represents a fatal condition due to its unfavorable location and biological behavior (1-3). Canine TCC is commonly located in the trigone region of the urinary bladder and may lead to partial or complete urethral obstruction. Complete surgical excision of TCC is often not possible due to its location and infiltrative growth pattern (2, 3). Common medical therapy of TCC consists of cyclooxygenase (COX) inhibitors and chemotherapy (2-4). Unfortunately most dogs that develop TCC ultimately die or are euthanized due to massive urethral infiltration (5). Local intralesional treatment with the cytokine interleukin-2 (IL-2) has been applied as a palliative treatment modality and has shown promising results (6). The purpose of this retrospective clinical study was to evaluate the influence of intralesional IL-2 treatment on the clinical course and tumor progression in dogs suffering from advanced urethral and urinary bladder cancer.

Correspondence to: Dr. med. vet. Ursula Konietschke, Small Animal Hospital, Keferloher Strasse 25, 85540 Haar, Germany. Tel: +49 8946148510, Fax: +49 894614851199, e-mail: ursula.konietschke@web.de

Key Words: Canine, transitional cell carcinoma, interleukin-2, urinary, bladder, urethral carcinoma.

Materials and Methods

Criteria for inclusion. Medical records of dogs presented at a private small animal referral clinic for intermittent lower urinary tract signs between 2003 and 2010 were retrospectively reviewed. Twenty-five dogs have been included in the study. Criterion for inclusion was the presence of a diffusely growing urethral or urinary bladder TCC, unfeasible for curative surgery.

Diagnosis. The diagnostic evaluation included physical examination, abdominal ultrasound, and cytological or histopathological examination of proliferative tissue in all dogs. Dogs with unremarkable bladder imaging underwent urethrocystoscopy. Tissue of sonographically visible intravesical masses was obtained by transabdominal ultrasound-guided fine-needle aspiration (FNA) for cytological examination.

Treatment. Ultrasound-guided intralesional IL-2 injection was performed in 14 dogs under intravenous propofol anesthesia, with an induction dose of 2-8 mg/kg. Irrespective of the animal's weight an overall dose of 18 million IU IL-2 (Proleukin®, Novartis) was injected with a 20-gauge needle, intralesionally, distributed at several locations. In four dogs with diffuse urethral carcinoma, the IL-2 application was conducted endoscopically or transrectally. Seven dogs underwent cytoreductive surgical tumor resection due to a more favorable tumor location. In the same session, IL-2 was administered to the tumor bed following partial cystectomy. In the course of partial cystectomy, unilateral ureterocystoneostomy was necessary in two dogs. Additionally, all dogs were treated with COX inhibitors as long-term medication. In 22 dogs, meloxicam was used at a dosage of 0.1 mg/kg, orally, every 24 h; piroxicam was administered at a dosage of 0.3 mg/kg, orally, every 24 h in two dogs; and firocoxib at a dosage of 5 mg/kg, orally, every 24 h in one dog. The chemotherapeutic agent mitoxantrone was used at the owners' request in two dogs at a dosage of 5 mg/m^2, intravenously, three times at three-week intervals.

Table I. *WHO clinical stages (TNM) of canine urinary bladder tumors.*

T	Primary tumor
T_{is}	Carcinoma *in situ*
T_0	No evidence of tumor
T_1	Superficial papillary tumor
T_2	Tumor invading the bladder wall with induration
T_3	Tumor invading neighbouring organs (prostate, uterus, vagina, anal canal)
N	Regional lymph node (internal and external iliac lymph node)
N_0	No evidence of regional lymph node involvement
N_1	Regional lymph node involved
N_2	Regional lymph node and juxta regional lymph node (lumbar lymph nodes) involved
M	Distant metastasis
M_0	No evidence of distant metastasis
M_1	Distant metastasis detected

Classifications. With regard to the tumor location, dogs were divided into three categories: those with tumors cranial to the trigone region (I), those with tumors in the trigone region and urethral involvement (II), and those with exclusively urethral involvement (III). A tumor-node-staging (TN-staging) was performed using the clinical staging system for canine bladder tumors, established by the World Health Organization (WHO) (Table I) (7). In terms of surgical intervention, the dogs were classified in two categories: those that underwent IL-2 treatment-exclusively (I), and those that underwent IL-2 treatment combined with cytoreductive surgery (II).

Follow-up examinations. Follow-up examinations were performed 2-4 weeks after IL-2 treatment. Further re-examinations were individually scheduled according to each animal's clinical course. In the present study, partial remission was defined as alleviation of the lower urinary tract signs and/or ultrasonographic regression of tumor size. Complete remission was defined as absence of ultrasonographically visible lesions and clinical signs.

Statistics. Survival times were calculated from the day of treatment until death, or the last day known to be alive. Dogs that were still alive were censored. Survival curves were drawn with the Kaplan Meier method. Tests for comparison of groups of survival data were performed using the log-rank test. The influence of age on survival was tested with the Wald test in univariate Cox regression. In terms of the variable age, differences between groups were evaluated with Students *t*-test or ANOVA test. For all analyses, values of $p<0.05$ were considered significant. The data were statistically analyzed using the software PASW Statistics Release 18.0.0, 2009 (SPSS Inc, Chicago, IL, USA).

Results

Animals. A total of 25 dogs, consisting of 19 females and 6 males were included in the study. The median age was 11.5-years, ranging from 8 to 14 years. There were 11 mixed breed dogs and 14 dogs of 13 different breeds. The dogs were presented at the small animal clinic between 2003 and 2010 due to the following lower urinary tract signs: hematuria (n=17), pollakiuria (n=13), dysuria (n=7), strangury (n=5), urinary incontinence (n=3), painful micturation (n=2). The major complaint in dogs suffering from urethral carcinoma, was acute dysuria.

Diagnosis and classifications. TCC of the trigone region with urethral involvement was diagnosed in 11 dogs. Eight dogs suffered from diffuse neoplastic infiltration of the urethra. Large, spreading diffuse lesions cranial to the trigone region were present in six dogs. TCC was diagnosed by in-house cytology. According to the WHO classification (Table I), 23 dogs were staged with T2, N0 disease; one dog with stage T3, N0 disease, due to tumor invasion into the pelvic canal; another dog was staged with T3, N1 disease based on ultrasonographically-visible invasion of neighboring structures and severe lymphadenopathy of the right external iliac lymph node. Intralesional IL-2 treatment without surgical intervention was performed in 18 animals. Medical management was chosen for dogs with non-resectable tumor localization. For seven dogs with tumor localization amenable to palliative surgical resection, IL-2 treatment was combined with cytoreductive surgery.

Clinical course. Evidence of adverse reactions associated with intralesional IL-2 treatment was not observed. At the first re-examination, 17 dogs were in partial remission. These animals exhibited normal micturation or distinct alleviation of lower urinary tract signs or ultrasonographic regression of tumor size. Four dogs which had undergone partial cystectomy were in complete remission. These dogs showed resolution of all clinical and ultrasonographic evidence of tumor. In three dogs, the disease was progressive; two of them suffered from urethral carcinoma. In the dog which was initially staged with T3, N0 disease, tumor invasion proceeded into the pelvic canal. One dog had stable disease, which meant unchanged findings at the time of the first follow-up examination. One dog developed a metastasis at the abdominal wall after seven months.

Survival times. The overall median survival time (MST) was 170 days (mean=301 days). The survival time ranged between

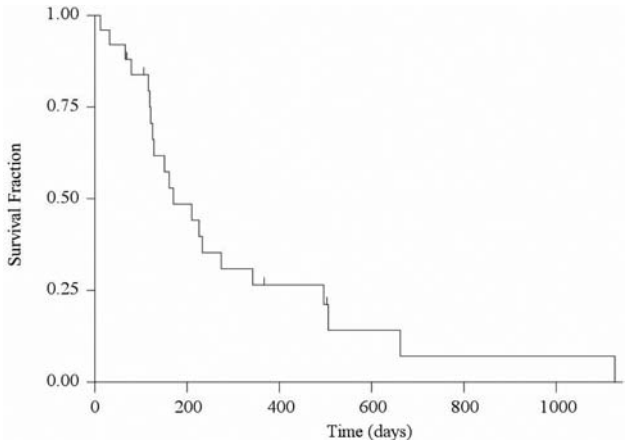

Figure 1. *Kaplan Meier curve of survival of 25 dogs with urethral or urinary bladder transitional cell carcinomas treated with intralesional interleukin-2. Vertical dashes represent censored data.*

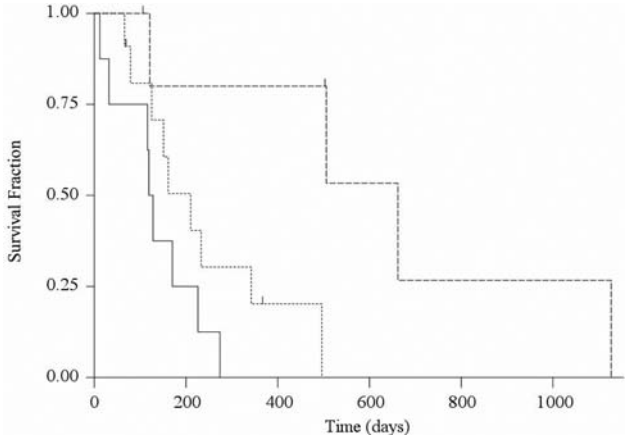

Figure 2. *Kaplan Meier survival curve of dogs with transitional cell carcinomas located in the urethra (solid line), in the trigone region (dotted line) and cranial to the trigone region (dashed line), respectively, treated with intralesional interleukin-2.*

12 and 1127 days (Figure 1). The gender did not have any statistically significant influence on the MST; the MST was 210 days in female dogs and 119 days in male dogs (p=0.354). Regarding the tumor location, there were statistically significant differences in MSTs (Figure 2). Dogs with carcinomas localized cranial to the trigone lived longest, with an MST of 662 days. The MST in dogs suffering from carcinomas localized in the trigone region was 210 days. The shortest MST of 119 days was observed in dogs with urethral carcinomas (p=0.006). Dogs which were excluded from cytoreductive surgery, due to unfavorable tumor location which were treated with intralesional IL-2 had an MST of 151 days (Figure 3). In contrast, dogs belonging to the surgical group had an MST of 506 days (p=0.008), even though only palliative cytoreductive surgery had been performed. Although statistically not significant, a possible correlation between age and survival time was noticed (p=0.069). There was no statistically significant correlation between age and method of treatment (p=0.424), gender (p=0.134) or tumor localization (p=0.413), respectively. At the time of study evaluation, four dogs were still alive, exhibiting none or only minimal problems with micturation.

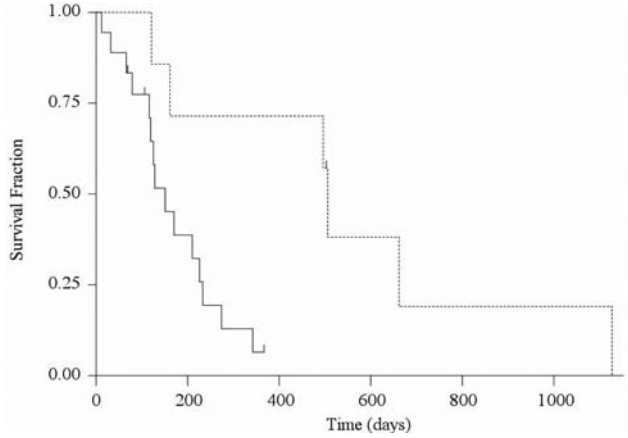

Figure 3. *Kaplan Meier curve illustrating the survival times of dogs treated with intralesional interleukin-2 (solid line), and those treated with intralesional interleukin-2 combined with cytoreductive surgery (dotted line), respectively.*

Discussion

TCC is the most common neoplasia of the urinary bladder in dogs (1-3). The tumor is most often located in the trigone region of the bladder. In a series of 102 dogs with TCC, the urethra was involved in 56% of dogs (2). In the present study, urethral involvement was observed at an even higher percentage (19 out of 25 animals; 76%). In about one third of the dogs (n=8), the carcinoma exclusively invaded the urethra, without ultrasonographically-visible tumor within the urinary bladder. Regarding the gender, multiple studies document an increased risk in female dogs (female-to-male ratio 1.7:1) (2, 3). In the present study, an even higher female-to-male ratio of 3.2 was found. The mean age of 11.5 years, calculated in our study is consistent with the reported mean age of 11.1 years at diagnosis (2, 3).

Complete surgical resection is often unfeasible due to an unfavorable localization and the malignant biological behavior of the TCC. In addition, many dogs appear to develop multifocal TCC in the bladder. In a series of 67 dogs with TCC that underwent surgery for biopsy and therapeutic intent,

complete surgical excision of the tumor was only possible in two animals (2). The inability to achieve wide resection margins is consistent with the findings in all dogs of our surgical group, in which resection margins were in close tumor proximity, precluding curative surgery. In two dogs, unilateral ureterectomy with subsequent neoanastomosis was necessary to achieve macroscopic resection of neoplastic tissue. According to the literature, radiation therapy is not considered to be a good treatment option as it is associated with a high incidence of complications (8). Studies in dogs with non-resectable or metastastic tumors support medical treatment using COX inhibitors alone or in combination with chemotherapy (2-4, 9). The MST of 62 dogs receiving the non-selective COX inhibitor piroxicam was 195 days and compared favorably to the MST of 109 days in 55 dogs which were treated with cytoreductive surgery-alone (2, 10). The efficacy of combined therapy using piroxicam and mitoxantrone has been evaluated and resulted in remission in 35% of dogs and an MST of 291 days (4). COX-2 is overexpressed in the majority of canine TCCs and is considered a major target of COX-2 inhibitor therapy (5, 11). Recently, antitumor activity against TCC was confirmed in a clinical trial in 26 dogs using the selective COX-2 inhibitor deracoxib (12). In this study, tumor response was determined by measurement of the primary tumor and metastases observed with ultrasonography and radiography. The MST was 323 days. Clinical signs were not used in categorizing tumor response, excluding the influence of clinical signs caused by urinary tract infection, which is in contrast to the present study. The majority of our dogs (n=22) received the selective COX-2 inhibitor meloxicam, which is believed to have similar antitumor activity in canine TCC. Comparing the MST of 170 days of the present study with that of 323 days of the previously mentioned study, the treatment applying a non-steroidal anti-inflammatory drug in combination with intralesional IL-2 injection does not seem to be superior to the single-agent treatment with a selective COX-2 inhibitor. These results may partly be explained by the different inclusion criteria. In the study of McMillan and colleagues (12), dogs with an expected survival of less than six weeks were excluded, which is in substantial contrast to that of the present study. Nineteen out of our 25 dogs suffered from carcinoma, localized exclusively in the urethra or at the trigone with urethral involvement. These animals were at danger of complete urethral obstruction and inevitable euthanasia, presumably within the following weeks. In previously mentioned studies, classification of exact tumor localization regarding urethral involvement was not performed, which is a substantial limitation comparing the clinical courses and MSTs. Regarding the present study, the main prognostic factor seemed to be the tumor localization with respect to urethral involvement. We conclude from our findings that urethral tumor invasion is the most important parameter to be evaluated regarding survival, even more important than TNM staging. In future studies, a clear definition of urethral involvement included in TNM staging would be appropriate.

In 17 out of 25 dogs, the owner subjectively noticed marked alleviation of lower urinary tract signs following IL-2 treatment. Owners observed either distinct relief or disappearance of hematuria, pollakiuria, dysuria and painful micturation in the dogs, which were classified as being in partial or complete remission. Intralesional infiltration with IL-2 in canine and feline, lower urinary tract carcinomas has already shown promising results in a pilot study (6). In that study, 13 dogs with histologically-confirmed TCC of the bladder and urethra received infiltration of IL-2, either endoscopically assisted or following surgical ablation of tumor tissue. Clinical improvement was observed in seven dogs. The MST was 210 days. The clinical response and survival results of that retrospective study approximate the observations of the present study.

In human medicine, local administration of IL-2 has been used for non-resectable TCC of the urinary bladder without observation of any adverse side-effects (13, 14). The therapeutic antitumor effect of local IL-2 treatment is induced by vascular leakage and consequently massive tumor necrosis, followed by stimulation of an immune response. In cases of a highly vascular neoplasia, tumor regression may be a rapid process, requiring about a week. In less vascular tumors, regression is caused by a cytotoxic leukocyte reaction and may require several months (15). Release of toxic granules from activated eosinophils as an antitumor effect of IL-2 has been proposed by Huland and Huland (16). Regarding the effect of IL-2 treatment, a wide individual difference in response was observed in the animals of the present study. The variable response to IL-2 treatment may be explained by individual immunological conditions and tumor state. Prospective trials implying an appropriate patient selection are necessary to compare the efficacy of (I) single-agent treatment with COX-2 inhibitors, (II) single-agent treatment with intralesional IL-2, and (III) combined therapy with COX-2 inhibitors and intralesional IL-2 application.

The limitations of the present study are rooted in the nature of retrospective studies. The definition of remission was mainly based on subjective clinical evaluation rather than on objective tumor measurement *via* ultrasound using defined urinary bladder volume. The time of re-examinations was not previously determined and varied individually.

Urothelial carcinomas were diagnosed in 22 dogs *via* cytological examination. Provided a well-experienced cytological specialist is available, cytological examination of samples gained by ultrasound-guided FNA biopsy represents a rapid and reliable modality of diagnosing TCC (17). An important discussion is the issue of localized tumor implantation of the ventral abdominal wall following percutaneous ultrasound-guided FNA biopsy (18). Despite

the rarity of needle-tract implantation, the consequence of this complication is not negligible and deserves consideration. Tumor-track implantation is a rare complication of FNA biopsy in humans, occurring at an estimated frequency of only 0.009% (19). The risk of needle-track implantation of TCC from FNA biopsy in dogs, however, is unknown (18). Some authors suggest traumatic urethral catheterization as the preferred modality to obtain material for cytological examination (5). Although neoplastic cells in the urine may be present in 30% of dogs, the differentiation between neoplastic cells and reactive epithelial cells associated with inflammation is difficult (20). In the present study, one mixed-breed dog developed an abdominal wall metastasis after seven months. For this dog, cytoreductive surgery was performed twice, including unilateral ureteral neoanastomosis. It remains undetermined whether the metastasis emerged as a result of the percutaneous FNA biopsy or because of the abdominal surgery. The advantages of transabdominal ultrasound-guided FNA biopsy include the minimally-invasive technique, which does not usually require anesthesia, expeditiously leading to diagnosis. Furthermore, ultrasound-guided FNA biopsy facilitates retrieval of representative tissue even in laterally localized, otherwise non-accessible lesions. If surgery is part of the therapeutic plan in an individual, resection of the needle track at surgery is recommended (18).

Conclusion

Intralesional IL-2 application is a safe and minimally-invasive palliative treatment option in symptomatic dogs suffering from diffuse widespread growing urinary bladder and urethral carcinomas, when surgical cure is impossible. The period of the palliative effect is highly variable. The prognosis depends on the tumor location and the feasibility of concomitant cytoreductive surgery. The results of the current study warrant further prospective randomized controlled clinical trials investigating the efficacy of intralesional IL-2 treatment for this often fatal condition.

References

1 Valli VE, Norris A, Jacobs RM, Laing E, Withrow S, Macy D, Tomlinson J, McCaw D, Ogilvie GK, Pidgeon G and Henderson RA: Pathology of canine bladder and urethral cancer and correlation with tumour progression and survival. J Comp Pathol *113*: 113-130, 1995.

2 Knapp DW, Glickman NW, DeNicola DB, Bonney PL, Lin TL and Glickman LT: Naturally occurring canine transitional cell carcinoma of the urinary bladder: A relevant model of human invasive bladder cancer. Urol Oncol *5*: 47-59, 2000.

3 Mutsaers AJ, Widmer WR and Knapp DW: Canine transitional cell carcinoma. J Vet Intern Med *17*: 136-144, 2003.

4 Henry CJ, MCCaw DL, Turnquist SE, Tyler JW, Bravo L, Sheafor S, Straw RC, Dernell WS, Madewell BR, Jorgensen L, Scott MA, Higginbotham ML and Chun R: Clinical evaluation of mitoxantrone and piroxicam in a canine model of human invasive urinary bladder carcinoma. Clin Cancer Res *9*: 906-911, 2003.

5 Knapp DW: Urinary bladder cancer. *In*: Current Veterinary Therapy XIV. Bonagura JD and Twedt DC (eds.). Philadelphia, Saunders Elsevier, pp. 369-373, 2009.

6 Nickel R, Eiermann C, Teske E, Zaal M, van den Ingh T and den Otter W: Local IL-2 treatment of lower urinary tract carcinomas in dogs. Anticancer Res *19*: 2009-2010, 1999.

7 Owen LN: Clinical stages (TNM) of canine tumours of the urinary bladder. *In*: TNM Classification of Tumours in Domestic Animals. Geneva, World Health Organization, p. 34, 1980.

8 Walker M and Breider M: Intraoperative radiotherapy of canine bladder cancer. Vet Radiol *28*: 200-204, 1987.

9 Knapp DW, Richardson RC, Chan TC, Bottoms GD, Widmer WR, DeNicola DB, Tecla WR, Bonney PL and Kuczek T: Piroxicam therapy in 34 dogs with transitional cell carcinoma of the urinary bladder. J Vet Intern Med *9*: 273-278, 1994.

10 Knapp DW: Tumors of the urinary system. *In*: Small Animal Clinical Oncology, 4th edition. Withrow SJ and Vail DM (eds.). Philadelphia, Saunders Elsevier, pp. 649-658, 2007.

11 Khan KN, Knapp DW, DeNicola DB and Harris RK: Expression of cyclooxygenase-2 in transitional cell carcinoma of the urinary bladder in dogs. Am J Vet Res *61*: 478-481, 2000.

12 McMillan SK, Boria P, Moore GE, Widmer WR, Bonney PL and Knapp DW: Antitumor effects of deracoxib treatment in 26 dogs with transitional cell carcinoma of the urinary bladder. J Am Vet Med Assoc *239*: 1084-1089, 2011.

13 Huland E and Huland H: Local continuous high-dose interleukin 2: A new therapeutic model for the treatment of advanced bladder carcinoma. Cancer Res *49*: 5469-5474, 1989.

14 Pizza G, Severini G, Menniti D, De Vinci C and Corrado F: Tumour regression after intralesional injection of interleukin 2 (IL-2) in bladder cancer. Int J Cancer *34*: 359-367, 1984.

15 Den Otter W, Jacobs JJ, Battermann JJ, Hordijk GJ, Krastev Z, Moiseeva EV, Stewart RJ, Ziekman PG and Koten JW: Local therapy of cancer with free IL-2. Cancer Immunol Immunother *57*: 931-950, 2008.

16 Huland E and Huland H: Tumor-associated eosinophilia in interleukin-2-treated patients: Evidence of toxic eosinophil degranulation on bladder cancer cells. J Cancer Res Clin Oncol *118*: 463-467, 1992.

17 Borjesson DL and DeJong K: Urinary tract. *In*: Canine and feline cytology: a color atlas and interpretation guide, 2nd edition. Raskin RE and Meyer DJ (eds.). St. Louis, Saunders Elsevier, pp. 249-259, 2010.

18 Nyland TG, Wallack ST and Wisner ER: Needle-tract implantation following us-guided fine-needle aspiration biopsy of transitional cell carcinoma of the bladder, urethra, and prostate. Vet Radiol Ultrasound *43*: 50-53, 2002.

19 Smith EH: Complications of percutaneous abdominal fine-needle biopsy. Review. Radiology *178*: 253-258, 1991.

20 Norris AM, Laing EJ, Valli VE, Withrow SJ, Macy DW, Ogilvie GK, Tomlinson J, McCaw D, Pidgeon G and Jacobs RM: Canine bladder and urethral tumors: a retrospective study of 115 cases (1980-1985). J Vet Intern Med *6*: 145-153, 1992.

Received June 1, 2012
Revised September 11, 2012
Accepted September 13, 2012