DISSERTATION

USE OF CONE-BEAM COMPUTED TOMOGRAPHY TO CHARACTERIZE URINARY BLADDER VARIATIONS AND OPTIMIZE DELIVERY OF RADIATION THERAPY FOR CANINE BLADDER CANCER

Submitted by

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ABSTRACT

USE OF CONE-BEAM COMPUTED TOMOGRAPHY TO CHARACTERIZE URINARY BLADDER VARIATIONS AND OPTIMIZE DELIVERY OF RADIATION THERAPY FOR CANINE BLADDER CANCER

Urinary bladder cancer is the most common cancer of the canine urinary tract, with transitional cell carcinoma (TCC) being the most commonly diagnosed tumor type. TCC is aggressive, invasive and fatal for most dogs. If left untreated, TCC of the canine bladder has average survival times less than one year.

Optimal treatment of this malignancy remains a topic of debate. Different treatment options exist, but many complicating factors make the probability of cure very low, regardless of treatment type, and most care is palliative in nature.

Radiation therapy is a possible treatment option, however dailyshape, size, and positional changes (motion) of the bladder and surrounding soft tissue structures often make this modality difficult to incorporate into a curative-intent treatment plan. This study was designed to investigate and quantify the motion characteristics experienced by the canine urinary bladder from day to day. Additionally, this information was then used to examine possible treatment scenarios and determine which of those scenarios would be optimal for canine

bladder cancer patients. Retrospective cone beam CT (CBCT) image data from ten dogs were used in this study. Organs of interest were contoured on each daily treatment CBCT data set and the images, along with the contours, were registered (fused) to the original (reference) planning CT. Quantification of bladder motion was determined by making measurements relative to the planning CT. Dosimetric data for the organs of interest were determined using dose volume histograms generated from sample treatment plans.

Results indicate a wide range in bladder motion throughout treatment, which partly depends on the methods used for patient positioning (set-up). Of the three patient positioning methods evaluated (dorsal, sternal, and lateral recumbency), the least amount of bladder variability, as well as lowest rectal dose, is seen when dogs are placed in lateral recumbency. Using these motion characteristics, we were able to develop different treatment planning and set-up scenarios that allow for a curative dose to be delivered to the bladder, while simultaneously reducing the dose delivered to the nearby sensitive rectal tissue. All advanced treatment planning techniques produce a better dose distribution than traditional parallel opposed planning, with adaptive radiation therapy (ART) planning techniques showing the most advantageous dose distribution.

These results allow for a more informed approach to the treatment of canine bladder cancer, as well as providing possible curative-intent treatment options for canine patients with this malignancy.

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CHAPTER 1
INTRODUCTION

1.1 Introduction

Over the past few decades, the use of external beam radiation therapy (RT) as a cancer treatment modality for companion animals has increased[1-3]. The technology involved in the RT process has evolved for both planning and delivery, and the number of facilities that are able to offer RT as a treatment option continues to increase[1, 4], with the Veterinary Cancer Society listing 69 clinics in 2010 that offer radiation therapy[4], up from 42 identified in 2001[1]. Technological advances in diagnosis, planning and treatment delivery have enabled RT to become an effective treatment option for most tumors, especially when combined with surgery[5]. Unfortunately, due to many contributing factors, this same success is not seen with all types of tumors. For instance bladder cancer, as evidenced by low survival rates when treated solely with RT[6], is still seen by many to be most effectively treated by cystectomy of the lesion, followed by chemotherapy. RT is oftentimes considered a reasonable palliative option[7] but geometric uncertainties in organ motion require increases in treatment margins which can increase the risk for normal tissue complications[8]. Thus, the use of RT for curative-intent treatment is often difficult. Such low survival rates and uncertainties in organ motion indicate that there is an opportunity for improvement when it comes to using RT as part of a multimodal, aggressive

curative-intent treatment option for urinary bladder cancer[8]. To make RT a curative, bladder-sparing alternative to cystectomy, some commonly encountered obstacles must be overcome. The goal of treatment planning is to increase the dose to the tumor while simultaneously limiting the dose to the surrounding tissues[9]. This introduces the possibility of acute and late effects on normal tissues surrounding the bladder [3, 6, 10-14]. In order to address these issues, the daily motion variations experienced by the canine bladder and surrounding tissues need to be understood[5]. Currently, there is little bladder motion data available and the majority is from human studies, many of these being gathered from prostate studies[8, 15 -25]. Using cone beam computed tomography (CBCT) imaging technology at Colorado State University Veterinary Teaching Hospital (CSUVTH) the geometric variations of the bladder were quantified and subsequently employed to develop RT protocols for the treatment of canine bladder cancer.

1.2 Canine Transitional Cell Carcinoma

1.2a TCC Incidence

Urinary bladder cancer is the most common neoplasm of the canine urinary tract, with the most frequently diagnosed type being transitional cell carcinoma (TCC)[26]. While the true incidence is not known, it is thought that between 20,000 to 30,000 dogs are affected by TCC each year in the United States[27], with the prevalence of all bladder cancers increasing over the last few

decades[28]. TCC is more common in certain breeds, including Scottish Terriers[28] (Table 1.1).

1.2b TCC Pathology

TCC is an invasive, progressive and ultimately fatal cancer that results in death due to post-renal obstruction within 3-12 months of diagnosis[29] if treatment is not administered. Most canine TCC tumor samples are intermediate to high grade, poorly differentiated and are of an infiltrative nature[26, 28]. At the time of diagnosis, many tumors have already invaded the muscular layers of the bladder.

Table 1.1 Breed and Risk of Developing Bladder Cancer[28]

Breed	Risk Factor
Scottish Terrier	18.09
Shetland Sheepdog	4.46
Beagle	4.15
Wire Hair Fox Terrier	3.2
West Highland White Terrier	3.02
Labrador Retriever	0.46
Golden Retriever	0.46

Risk factor for each breed when compared to mixed-breed control dogs.

1.2c TCC Causes

The etiology of bladder TCC is not known, but is most likely due to multiple factors[28]. It is suspected that a combination of genetic and environmental causes, such as exposure to insecticides, obesity, sex and breed, contribute to the development of this malignancy[27-30]. It has been demonstrated that cigarette smoke, occupational chemical exposure and insecticides are powerful causal agents in the development of human bladder cancer[30], and this holds true for canine patients as well. Exposure to insecticides and herbicides has been shown to increase the risk of a dog developing TCC of the urinary bladder[28-30]. A study of 58 cases showed that dogs exposed to insecticides had double the risk of control dogs of developing bladder TCC[30]. Many of these products are often petroleum-based, which have been identified as a risk factor for human bladder cancer[30].

In the above study, obese dogs had a higher incidence of bladder TCC than did the control dogs[30], and this was attributed to the lipophilic nature of many chemicals which become stored in the dog's adipose tissue[28, 30].

Studies have also shown the increased incidence of bladder cancer in female dogs. In a series of 102 dogs, female dogs were treated for bladder TCC 1.7 times as often as male dogs[28]. Higher incidence in female dogs has been attributed to the higher percentage of body fat in female dogs versus male dogs, which acts to sequester the lipophilic chemicals in the insecticides[28, 30]. Female dogs urinate less frequently than male dogs which would result in less carcinogen-exposure time for the male dogs[28]. Breed-associated risk is likely

genetic[28] and may be attributed to different pathways that activate or detoxify different carcinogens, such as benzene, which is a common ingredient in many insecticides[28, 29].

1.2d TCC Diagnosis

Clinical signs of TCC include incontinence, difficulty urinating, pollakiuria, and hematuria. Signs of renal failure (vomiting, anorexia, dehydration) may occur, as well as urethral or ureteral obstruction[29], in advanced cases. Diagnosis begins with either cytologic diagnosis from urine sediment cytology or intentional urinary tract catheterization, or histopathologic examination of a tissue sample or biopsy through cystotomy, cystoscopy or catheter biopsy[31]. Staging is then performed through physical examination, radiologic imaging of the thorax, bladder ultrasound, and abdominal radiography, ultrasound, and/or CT to further asses the location of the tumor and disease extent.

Approximately 37% of dogs show metastatic disease at the time of diagnosis[26, 28] and metastatic disease is reported in approximately 50% of cases at time of death [26, 29, 32]. Sites of bladder TCC metastases in a postmortem examination of 50 dogs included lung (28%), regional lymph nodes (26%), liver (18%), kidney (4%), spleen (4%), prescapular lymph nodes (4%) and uterus (4%) one case each (2%) of metastases in mesenteric lymph nodes, cecum, bronchial lymph nodes, vertebrae, ilium, colon, abdominal wall, diaphragm, renal lymph node, and oral mucosa[28]. Death due to urinary tract obstruction often occurs prior to the development of a lethal metastasis when the primary tumor is not

controlled. Death due to metastatic disease occurs more often if the primary tumor can be controlled[28].

1.3 Treatment Options

Treatment options depend on the location and invasiveness of the tumor and include surgery, chemotherapy, and radiation therapy, or some combination of these modalities[12]. Unfortunately, a unique combination of factors, as well as a low probability of cure, makes TCC difficult to effectively treat and most cases are treated with palliative, rather than curative, intent[32] (Table 1.2). Prognosis is often worse with a younger age of onset, more invasive tumors, and tumors that also involve the prostate gland.

1.3a Surgical Excision

Surgical excision without adjuvant therapy is not considered curative. Furthermore, surgical excision is not often considered a treatment option due to the frequent location of tumors in the trigone area or urethra[28,33], the possibility that there are multifocal lesions that differ in aggressiveness[34], and the concern of tumor seeding[32, 35-37]. Local disease is often advanced and involves the muscular layers of the bladder and urethra[26,34]. Invasive tumors involving the muscular layers of the bladder also require more radical surgeries, to which owners may not agree due to the possibility of side effects, such as urinary incontinence and increased urinary frequency[28]. Partial cystectomy has been reported, but tumor recurrence occurred in 8 of the 10 dogs in the study[33].

Recurrence is also thought to occur in up to 70% of human cases of bladder TCC[38]. Median survival times for canine patients have been reported at just over 100 days when surgical debulking, with or without partial cystectomy, is the only treatment[26].

Another concern that prevents surgical excision from being an effective single-agent effective treatment option is the thought that the entire bladder mucosa has likely been exposed to the inciting carcinogen as it is metabolized and exits the urinary tract in the urine[7]. New lesions occurring after surgery are often noticed at sites distant from the surgical site[28].

1.3b Medical Therapy

Chemotherapeutic agents are often used to treat canine bladder TCC due to the aggressive nature of the disease and the high metastatic rate[32], however an effective protocol has not been established. Platinum-based drugs (cisplatin, carboplatin, etc.) and non-steroidal anti-inflammatory drugs (NSAID) (cox inhibitors, piroxicam) have been used with only moderate success[32]. While single agent chemotherapy has been shown to help prolong or improve the quality of the dog's life, it is not curative in most cases and the toxicity associated with more aggressive therapies are often not tolerated by owners[32].

Piroxicam has been shown to provide both anti-cancer and analgesic benefits, however it carries with it the possibility of gastrointestinal toxicity[29, 39, 40]. Piroxicam has been used with some success and has been reported to produce measurable tumor shrinkage, however survival times when used as the sole

treatment show median survival times of only about six months. More recently, combined therapy using cox-inhibitors, such as piroxicam, with mitoxantrone, has been shown to provide both anti-cancer and palliative benefits[41], with results that are superior to using single agent chemotherapy.

1.3c Radiation Therapy

Radiation therapy (RT) is a bladder-sparing alternative to cystectomy, although the possibility of acute and late radiation effects limits its use[10, 32]. There are few reports of it being used to successfully treat canine TCC and RT alone is often considered inferior to cystectomy, in terms of survival[8]. In human medicine, RT is used in patients with unresectable or inoperable tumors to preserve bladder function[10]. In veterinary medicine, it is considered a reasonable palliative treatment option to relieve pain or adjuvant treatment option[7], but organ motion, the need for large margins and the possibility of injury to adjacent structures limit curative-intent treatment of intra-abdominal tumors with external beam RT[42, 43].

One of the major obstacles that prevent RT from being a curative treatment option is the daily possibility of variations in size, shape and location of the bladder and surrounding soft tissue structures[8, 15-20]. Further complicating RT delivery is the difficulty in visualizing such soft-tissue variations using standard megavoltage (MV) portal imaging techniques (Fig. 1.1). These uncertainties require larger treatment margins to ensure coverage of the bladder each day, but can lead to side effects from irradiation of neighboring dose-limiting structures,

including the rectum[8, 12]. Historical side effects associated with canine bladder radiation therapy have included urinary incontinence, cystitis, pollakiuria, and stranguria. Tumor control can be compromised if the dose is decreased to spare the adjacent tissues[8].

Table 1.2 – Average Survival Times with Different Treatment Options

Treatment	Median Survival
None	< 6 months
Surgery	125 days[26]
RT	< 1 year
Surgery + RT	450 days[44]
Chemotherapy + Piroxicam	9-12 months[41]
Piroxicam/NSAID	195 days[39]

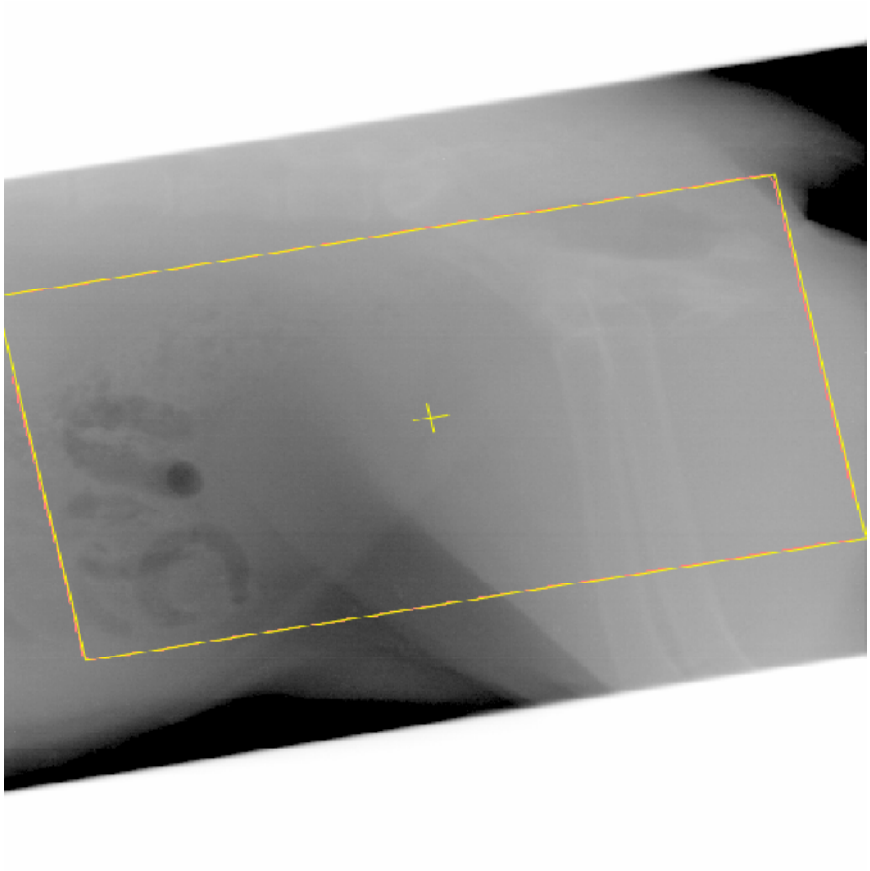


FIG. 1.1 Example of a MV portal radiograph typically used for patient set-up. Right lateral view of canine abdomen in left lateral recumbency. Little information about the daily variations in the bladder and surrounding soft tissue structures is available using this imaging modality.

Normal internal physiological movement of the urinary bladder and bowel causes daily variations in location[8, 15-20]. If organ movement is not accounted for in the treatment volume, or the planning target volume (PTV), geographical miss is likely to occur, resulting in reduced local tumor control. Typically, the treatment margins are increased to compensate for the size and shape of the bladder and uncertainty of its location[45]. Increasing the irradiated margins also increases the possibility a critical structure, such as the colon, will be irradiated beyond tolerance[22, 43], leading to unacceptable complications, including colitis, strictures and bowel perforation[11, 32, 46]. The prescription dose is often lowered in an effort to limit the side effects of irradiating nearby sensitive structures, which may result in the bladder receiving a non-curative dose.

Despite advances in RT treatment technology, little data is available describing the daily, interfractional motion characteristics of the bladder, especially in veterinary patients. Most organ motion data has been based on human prostate studies[22]. Only a few studies have focused on bladder motion and bladder motion is hypothesized to be much greater than that of the prostate, due to its anatomical characteristics.

1.4 Radiation Biology and Fractionation

Ionizing radiation causes cellular damage in multiple ways. Direct effects occur when secondary electrons interact directly with and damage the cellular DNA. Indirect effects occur when the secondary electrons interact with water molecules and produce free radicals. Free radicals are unstable and can either

revert back to their original form, or other free radicals, or react with oxygen molecules in oxygenated cells to produce peroxides. Peroxides may cause irreparable damage to the chemical structure of the cell. Indirect methods are responsible for approximately two-thirds of the biological damage to cells from x-rays[9].

Irradiated cells typically die attempting their next cell cycle or during their next mitosis, but can take up to 5 mitoses[9]. The damage caused by the peroxides is often the cause of this cell death. However, if the cells are hypoxic, these peroxides are not formed as readily and the cells can repair themselves. Hypoxic cells are two to three times more radiation resistant than oxygenated cells.

At any given time, there is a population of cells in the tumor that are not well oxygenated and will not respond to irradiation. The total radiation dose is broken into smaller doses, or fractions, and delivered over time to combat this. Fractionation allows for a differential response of tumor and normal tissue cells. Normal tissues are given the chance to repair between fractions. Thus, administration of radiation in small doses per fraction preferentially spares late responding normal tissues. Tumor cells become reoxygenated and redistribute into a different phase of the cell cycle that are more radiosensitive.

1.5 Acute and Late Radiation Effects

Regardless of the type of RT or the manner in which it is delivered, a common goal is to deliver the highest total dose possible to achieve tumor

control while simultaneously sparing any nearby critical structures[9]. During RT for bladder cancer, normal tissues such as colon, rectum, urethra, bone, small intestine, and spinal cord are included in the radiation field, which puts them at risk for the development of acute and/or late effects[11, 12, 47]. It is the possible toxicity to these critical structures that dictates the limiting dose that can be given to the PTV[6].

Acute effects are most often observed within 10-14 days after the start of treatment, may persist throughout the course of treatment, and most often subside within 2-3 weeks after treatment has ended[9]. Such side effects include radiation dermatitis, acute colitis, proctitis, enteritis, and cystitis/urethritis[8, 11, 48-50] (Table 1.3). They are most certainly uncomfortable for the patient and require extra care by the owner and veterinarian. However, they are rarely life threatening and usually resolve with appropriate care, such as anti-inflamatory drugs, antibiotics and pain management[3, 11-13]. Acute effects are rarely dose-limiting[3, 11, 12].

Late effects occur months to years after a course of RT has been given and are dose limiting[9]. They are caused by damage to the parenchyma and connective tissues and are irreversible, often progressive, and can negatively impact the quality or length of the patient's life[11, 12]. They can arise directly or can be the result of severe radiation injury to acutely-responding tissues, known as consequential late effects[9]. Late effects seen with bladder cancer RT are chronic colitis, proctitis, gastrointestinal perforation, rectal and anal fistulas, strictures (urinary, rectal, and gastrointestinal), and bone necrosis[11, 12] (Table

1.3). Studies have shown that the number and severity of late effects increases with increasing dose per fraction above 2.7 Gy[11, 12].

Table 1.3 Acute and Late Effects of Canine Pelvic Irradiation

Acute Effects	Late Effects
radiation dermatitis	chronic colitis
acute colitis	chronic enteritis
acute proctitis	chronic proctitis
acute enteritis	gastrointestinal perforation
cystitis/urethritis	gastrointestinal stricture
	urinary bladder fibrosis
	myelopathy
	bone necrosis

1.6 Radiation Therapy Protocols

1.6a Standard CT-Based Bony Anatomy Protocol

There is a wide diversity of external beam RT treatment devices used in veterinary facilities, but the trend over the last few decades has been towards the utilization of Cobalt-60 units and linear accelerators[2]. Cobalt-60 units utilize gamma-rays and linear accelerators utilize x-rays, but both types of devices deliver megavoltage (MV) therapy. Cobalt-60 units have an average energy of approximately 1.2 MeV from gamma radiation and a D_{max} of 0.5 cm. Medical linear accelerators typically produce bremsstrahlung x-ray spectra from 4 – 25 MV, with D_{max} for a 6 MV unit at 1.5 cm.

The typical planning process begins with the patient receiving an original planning CT scan with the patient in the treatment position and using any immobilization devices required for a reproducible daily set-up. The planning CT is then transferred to a treatment planning system (TPS) where the tumor is located and organ contours are defined. Other volumes of interest are defined as required based on the location and extent of the tumor. The gross tumor volume (GTV) (Fig. 1.2, red contour) is the visible or palpable tumor mass that can be visualized by imaging techniques, such as on the planning CT. The GTV, plus an added margin to account for any microscopic or subclinical disease, is defined as the clinical target volume (CTV) (Fig. 1.2, yellow contour). The CTV is defined as the entire bladder in most bladder cancer cases. The CTV, plus an added margin to account for set-up errors and inter- and intra-fraction organ

motion, is defined as the planning target volume (PTV) (Fig. 1.2, green contour). The PTV is the radiation target volume. Critical organs, such as the rectum, are defined in the RT planning process in order to avoid them in the radiation plan in addition to the radiation target volume.

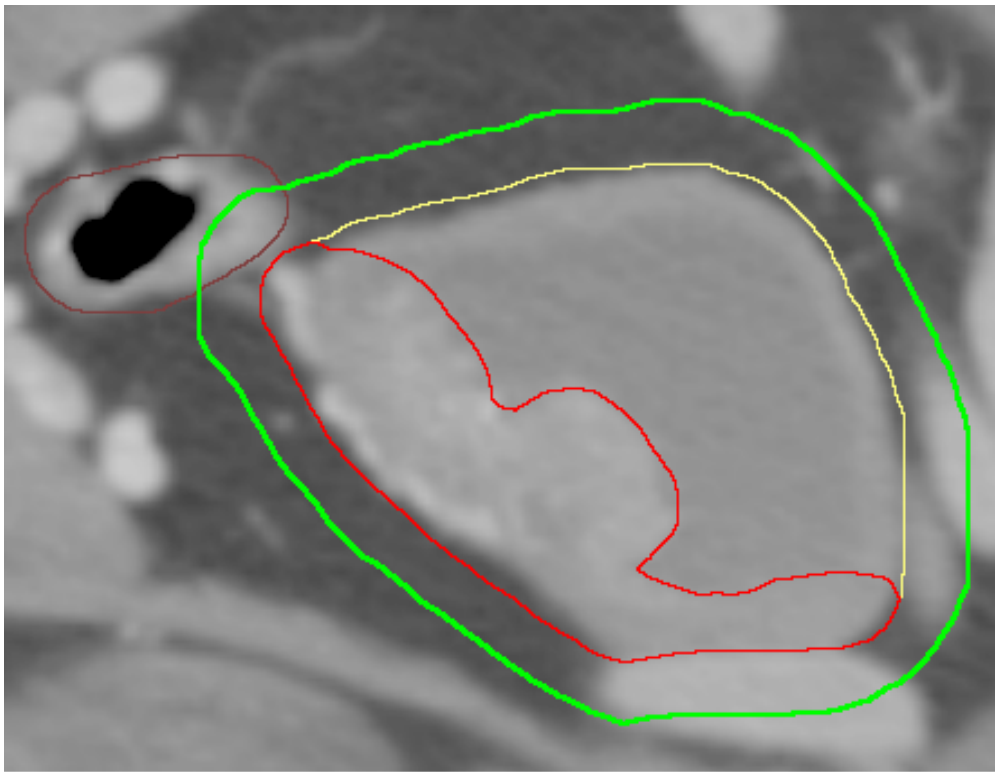


FIG. 1.2 Contours drawn on a planning CT to define the GTV (red), bladder CTV (yellow), PTV (green), and the structure to avoid, the rectum (brown).

All volumes and organs of interest are contoured on the planning CT and a radiation plan developed. A survey of veterinary radiation therapy facilities found that canine bladder protocols range in prescription from 2.25 to 3.2 Gy/fraction daily, Monday through Friday, for 16-25 fractions with a total dose of 48-63 Gy to be delivered to the PTV[1, 11]. Palliative protocols use larger doses per fraction, and are delivered less often.

Dogs are anesthetized and positioned for daily treatment in the same position as for the planning CT, utilizing the same immobilization and set-up devices, as required. Patient position is verified via radiographic imaging, typically portal radiographs[2, 51]. The daily position of the patient is aligned to match the position during the planning CT utilizing the patient's bony anatomy landmarks or implanted fiducial markers, as seen on the radiograph. Once the patient position is verified, the prescribed plan is delivered to the PTV.

1.6b CT-Based Soft Tissue Protocol at CSU

Currently at CSU, the original planning CT scans for patients are obtained using a multislice helical scanner utilizing 2 mm thick slices while the patients are in the treatment position. Planning CT's are acquired at approximately the same time the dogs will be treated each day in order to standardize bladder and rectum sizes. Dogs are allowed to void their bladders in the morning, but defecation is not allowed until after treatment. Morning food intake is prohibited until after treatment.

Planning CT images are transferred to a Varian Eclipse TPS. IMRT plans are constructed using the entire bladder volume as the CTV. Margins from 5-10 mm are typically added to the CTV to construct the PTV, which becomes the target volume. Multi-leaf collimators (MLC) are used to achieve field shaping and dose conformality.

Cone beam CT (CBCT) images are acquired immediately prior to each daily treatment session using the Varian Trilogy On-Board Imaging (Varian Medical Systems, CA, USA) kV X-ray source and digital detection panel mounted on the gantry of the linear accelerator, instead of portal radiographs. The CBCT acquires 3D images of the patient's internal anatomy at 125 kVp and 80 mA. Each daily CBCT images the portion of the abdomen containing the bladder and can be reconstructed with slice thicknesses ranging from one to 10 mm. Two mm slices were used with a 512 × 512 reconstruction matrix for this study. The CBCT images are used to characterize the bladder each day and make patient positioning adjustments. CBCT allows for visualization of the bladder each day and the patient is able to be positioned based on the daily bladder position instead of being based on bony anatomy. The prescribed dose is delivered to the PTV after the patient is set up appropriately.

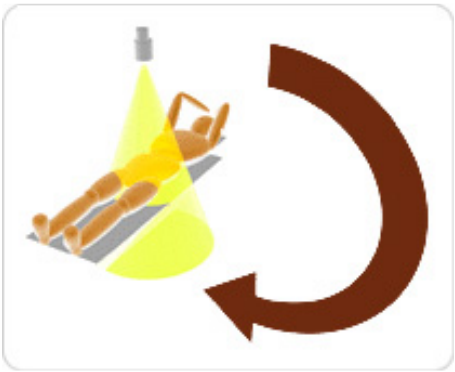
1.7 Cone Beam Computed Tomography

Increasing complexity of RT treatment plans and the ability to deliver more conformal doses with techniques such as 3D conformal radiation therapy (CRT) and intensity modulated radiotherapy (IMRT) increases the importance of delivering the planned dose accurately. One of the newer imaging modalities to

help achieve accurate delivery is kilovoltage (kV) cone beam computed tomography (CBCT). CBCT is comprised of a single, cone-shaped x-ray beam on one arm of the linear accelerator and a flat panel detector on the other. The arms rotate 360° around the patient one time to acquire the image volume (Fig. 1.3).

Images are constructed using an algorithm that allows viewing by slice, similar to a traditional CT. Image quality is slightly inferior to a traditional CT because the unique geometry of CBCT introduces more scatter which slightly reduces contrast and increases noise on the image[45]. Despite the increased noise, soft-tissue and bony structures are easily visible (Fig. 1.4) and the CBCT images can be used to locate structures of interest and assist in patient positioning.

CBCT is a valuable tool in the imaging of volumetric soft tissue anatomy for patient positioning and target verification[45]. CBCT improves geometric accuracy for advanced treatment delivery and allows for an increase in dose to tumor while sparing normal tissues adjacent to the treated volume.



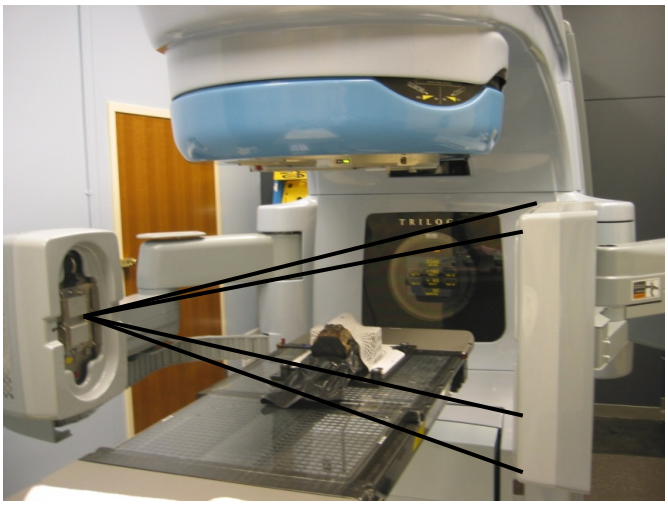


FIG. 1.3 Cartoon depicting CBCT rotation around patient (top) and photograph of gantry-mounted CBCT with model patient in treatment position (bottom).

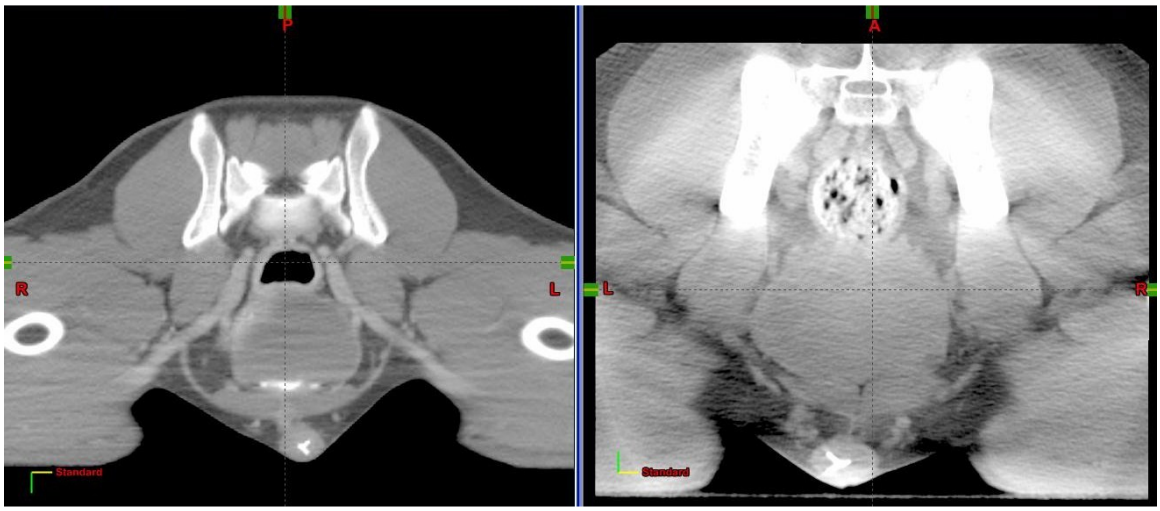


FIG. 1.4 Traditional CT (left) shows higher image quality than CBCT (right) due to scatter, however structures of interest are easily visible with CBCT for positioning and target visualization.

1.8 Study Aims

Many unique factors regarding canine urinary bladder cancer necessitate the treatment of the entire bladder. Different treatment options exist for this malignancy; however treatment outcomes are often less than desirable regardless of treatment type. RT has been no exception.

Motion of the bladder and surrounding pelvic organs, as well as deformation of the bladder due to differing states of filling, are the dominant sources of error in the planning and delivery of RT for canine bladder cancer. Use of non-optimal margins to account for this uncertainty compromises patient care and adversely affects treatment outcome. No data is available describing the daily motion and position characteristics of the canine bladder to date.

The hypothesis of this study was twofold. We first hypothesized a quantification of the daily motion characteristics of the canine urinary bladder could be accomplished using retrospective patient data acquired through daily CBCT imaging unique to CSU. The bladder is one of the sites with great potential for benefit from imaging technology, providing daily visualization of the target prior to treatment[52]. We next hypothesized that bladder motion and position data could be used to develop an optimal RT protocol that would deliver a conformal, curative-intent dose to the bladder while minimizing the dose received by the nearby rectum. A unique study aim was used to address each hypothesis.

1.8a Aim 1 – Characterization of Interfractional Bladder Variations

Aim 1 of this study addressed our hypothesis that we could use CBCT imaging technology to:

1. quantify the daily motion characteristics of the canine urinary bladder in three different treatment positions, and
2. recommend an adequate PTV expansion margin that would ensure adequate irradiation of the entire bladder each day, while minimizing unacceptable complications due to irradiation of the rectum.

Quantification of the daily bladder wall position variations experienced by the canine urinary bladder on a daily basis with dogs in three possible treatment positions was done by making distance measurements on retrospective daily CBCT images. The data from these measurements was then used to determine the most advantageous treatment position for canine bladder cancer patients receiving external beam RT. The most advantageous position was that which showed the smallest amount of bladder wall variation in six measured directions.

A 5, 10 or 15 mm treatment margin was added to the planning CT bladder volume, in addition to taking bladder variation measurements. Sample two-field plans using parallel opposed beams were developed that utilized actual, retrospective daily positioning images. Dosimetric data for each plan was then examined using the dose volume histogram (DVH) generated by the TPS. The recommended PTV expansion would be the volume that ensured adequate

irradiation of the entire bladder each day while minimizing the dose received by the rectum.

1.8b Aim 2 – Optimization of Bladder Cancer Radiation Therapy using Bladder Motion Data

Aim 2 of this study addressed our hypothesis that we could use the bladder motion data from Aim 1 to:

1. develop plans and compare dosimetric data for different advanced RT techniques, and
2. develop an adaptive RT (ART) protocol that would optimize the dose delivery for canine bladder cancer.

The bladder motion and treatment setup data from Aim 1 was used to create different types of advanced RT plans. Advanced plan types evaluated included an intensity modulated RT (IMRT) plan using bony anatomy registration and an IMRT plan using soft tissue registration. The feasibility of an ART plan that used a new target volume based on each day's anatomy was also examined. The dosimetric data for the new plans, as well as the dosimetric data from the parallel opposed plans in Aim 1, was compared using DVH's. The optimal treatment protocol was selected to be the plan that provided the most conformal, curative-intent dose to the bladder while simultaneously minimizing the dose to the rectum, as determined by dose volume histogram (DVH).

CHAPTER 2
CHARACTERIZATION of INTERFRACTIONAL BLADDER VARIATIONS and IMPACT of PLANNING TARGET VOLUME EXPANSIONS

2.1 Introduction

2.1a Implications of Non-optimal PTV Margins

Organ motion data is an integral part of effectively planning RT for a patient, however most studies that have addressed this issue for pelvic irradiation have been human prostate studies. Motion of the prostate, while affected by motion of the organs surrounding it, is not affected by filling, unlike the bladder. The urinary bladder is a hollow organ that varies in position due to pressure from other organs in the pelvis and changes in urine filling of the bladder itself. Both external pressure from surrounding organs and volume changes of the bladder may occur simultaneously, resulting in substantial movement of the bladder[8, 53]. In addition, tumors experience intrafractional motion during a treatment session, including normal peristaltic motion of the digestive tract.

In order to effectively treat canine bladder tumors, the characteristics of interfractional bladder variations need to be understood. A routine practice is to add a safety margin around the target volume to reduce the risk of a geographic miss however, this may result in unnecessary irradiation of the surrounding critical tissues[47]. Interfractional bladder variability information is a crucial component of defining an appropriate treatment margin. If the daily bladder

variation is greater than accounted for with the PTV, the prescription dose to the CTV may not be achieved and tumor control will be compromised. If the daily bladder motion is much less than accounted for with the PTV, the tolerance dose to the normal tissues may be unnecessarily exceeded[5, 8]. Currently, it is not clear what PTV margins should be used in clinical practice for the treatment of canine bladder cancer.

2.1b Treatment Positions

Different methods have been used to deliver an effective dose while minimizing colon and rectum irradiation to lessen the morbidity associated with bladder RT. One method used with some success in both human and canine patients is surgically-implanted tissue expanders. Studies have reported fewer radiation-induced side effects and lower percentages of bowel in the radiation field [42, 54-57]. Tissue expanders create physical separation between the bladder and rectum, thus providing a margin that has the potential to allow delivery of higher total doses without adverse side effects to the colon[42].

We hypothesized that optimal choice of patient positioning on the treatment couch would improve physical separation of the bladder and rectum without the risks of infection from invasive surgery. The appropriate treatment position was investigated as a technique to ameliorate the negative impact of bladder motion and large PTV margins by allowing the bladder and rectum to naturally separate.

Three different patient positions were chosen for evaluation. The CSU standard of care was to treat bladder and prostate cancer IMRT patients in dorsal recumbency upon study initiation (Fig. 2.1, top). Dorsal position images were obtained from patients treated using this initial treatment setup. The standard of care at CSU was changed to treat patients in sternal recumbency during the study (Fig. 2.1, middle). The hypothesis was that the treatment couch adjacent to the abdominal wall would allow for a more reproducible treatment set-up from day to day by preventing variation in the ventral bladder wall. Sternal position images were obtained from patients using this treatment setup. The hypothesis was that lateral recumbency (Fig. 2.1, bottom) would provide physical separation and an easier treatment set up, and preliminary data from this study showed that to be the case. Lateral recumbency became the standard of care for bladder and prostate cancer IMRT at the CSUVTH. Lateral position images were obtained from patients using this treatment setup.

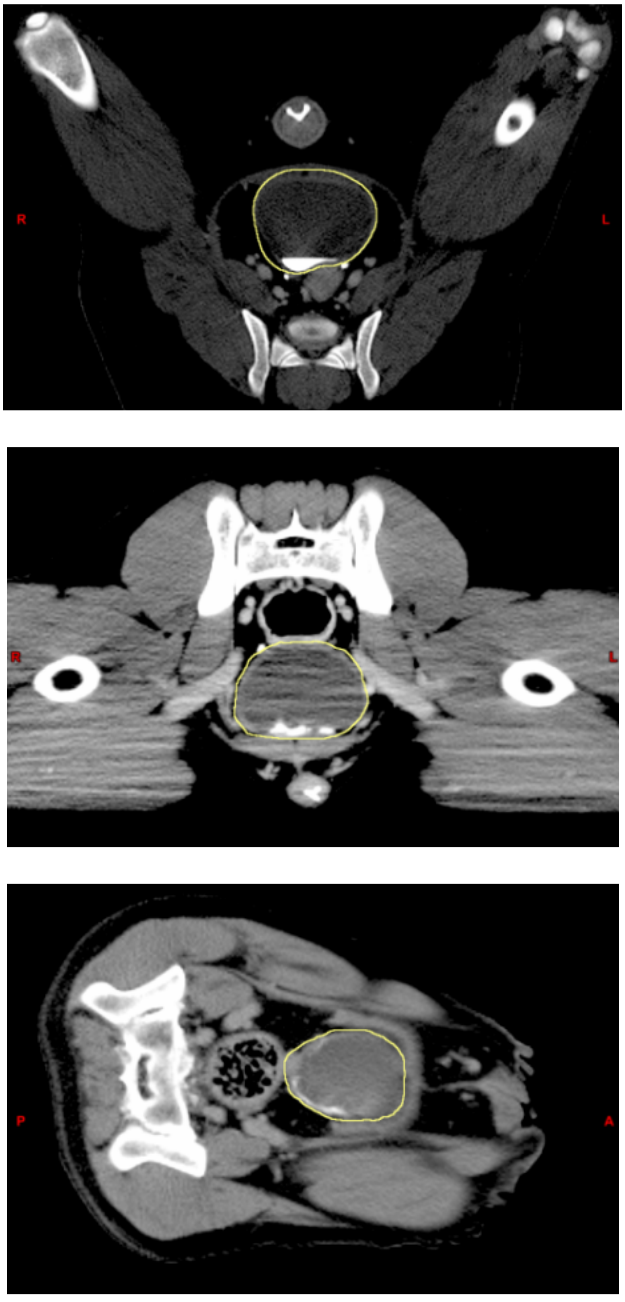


FIG. 2.1 Treatment positions evaluated. Dorsal recumbency (top), sternal recumbency (middle), and lateral recumbency (bottom).

2.2 Materials and Methods

2.2a Patient and Image Selection

Subjects for this study were client-owned canine patients undergoing standard-of-care fractionated intensity modulated RT (IMRT) at the CSUVTH for bladder or prostate cancers. Images from patients undergoing treatment for either disease were used because the organs of interest for this study (bladder and rectum/colon) are visible in the images of both types of cases. This study did not alter patient treatment and only retrospectively evaluated image data that was collected for patient positioning.

The original planning CT scans were obtained using a Picker PQ2000 (Picker Medical Systems, Cleveland, OH, USA) CT helical scanner with two mm thick slices while the patients were in the treatment position. A vacuum-shaped cushion was formed around the patient while in the original position in order to reproduce this position each day thereafter, and it was then indexed to the treatment couch in the same location for each subsequent treatment.

The cone beam CT images were acquired immediately prior to each daily treatment with the patient in the treatment position using the Varian Trilogy On-Board Imaging (Varian Medical Systems, Palo Alto, CA, USA) kV X-ray source and detection panel mounted on the gantry of the linear accelerator. The CBCT makes one complete 360-degree rotation around the patient and acquires 3D images of the patient's internal anatomy using a technique of 125kVp and 80 mA. Each daily CBCT visualizes the portion of the abdomen containing the bladder and can be reconstructed with slice thicknesses ranging from one to ten mm.

Two mm slices were used with a 512 × 512 reconstruction matrix for this study. In an attempt to standardize bladder and rectum sizes on a daily basis, dogs were treated at approximately the same time each day as the planning CT was acquired. Food intake was prohibited until after treatment. Dogs were taken outside to allow them to void their bladders prior to being anesthetized, however they were not allowed to defecate at this time. This allowed a small amount of stool to be present in the rectum, as it aids in treatment setup and delivery

Images from ten dogs were available for this study. Three dogs were in dorsal recumbency, four dogs were in sternal recumbency and three dogs were in lateral recumbency. Fourty four images were daily dorsal CBCT's (mean = 15 images per dog), 37 were daily sternal CBCT's (mean = 9 images per dog), and 29 were daily lateral CBCT's (mean = 9 images per dog).

2.2b Contouring

All planning and CBCT images were transferred to the Varian Eclipse Treatment Planning System (TPS), software version 8.6.15. The bladder was contoured on each slice of each daily CBCT to include the entire volume encompassed by the bladder wall, including the bladder contents, as well as the trigone and 2 cm of the urethra caudal to the bladder. The transverse colon was the cranial boundary. The length of the rectum adjacent to the bladder was also contoured and included the entire rectal volume and its contents encompassed by the outer rectal wall, from the sigmoid flexure cranially to two cm past the

urinary bladder caudally. All organ contours were drawn on each slice by hand by the same investigator (JRN) to ensure consistency.

2.2c Bony Anatomy Registration

The CBCT data sets were registered to the corresponding original planning CT image set using bony anatomy as landmarks, such as the spine and pelvis using the Varian rigid registration algorithm. The algorithm uses CT pixel values and provides the option for manual adjustment as needed for the bony anatomy registration. All bladder volumes contoured on the daily CBCT scans were copied to the original planning CT and in the same planning CT bony anatomy frame of reference. Bony anatomy registration allows for the measurement of variations in position of each daily bladder volume with respect to the original bladder volume contour (defined on the planning CT).

2.2d Bladder Variation Measurements

The urinary bladder is a hollow organ and there is the possibility that each wall can move independently[8], so measurements were taken in the right, left, dorsal, ventral, cranial, and caudal directions. All measurements were taken relative to a single constant reference point that was determined from the planning CT (Fig. 2.2). This reference point was placed at the 3D center of mass of the bladder volume as defined on the planning CT. Once a point was defined for each patient, it remained constant for all subsequent measurements of bladder wall position for that patient.

Measurements were made from the reference point in each of the six directions to the bladder wall's maximum displacement in that direction (Fig. 2.2). The entire bladder volume was considered, not simply each slice, to define a maximum displacement in one of the measured directions. To account for variations in dog size between different patients, daily positions of the bladder wall were compared to the bladder wall position on the day of the planning CT. The absolute displacement of the bladder wall from the reference point for each day was divided by the original bladder wall displacement from the reference point to provide a percentage-change in bladder wall position for each day.

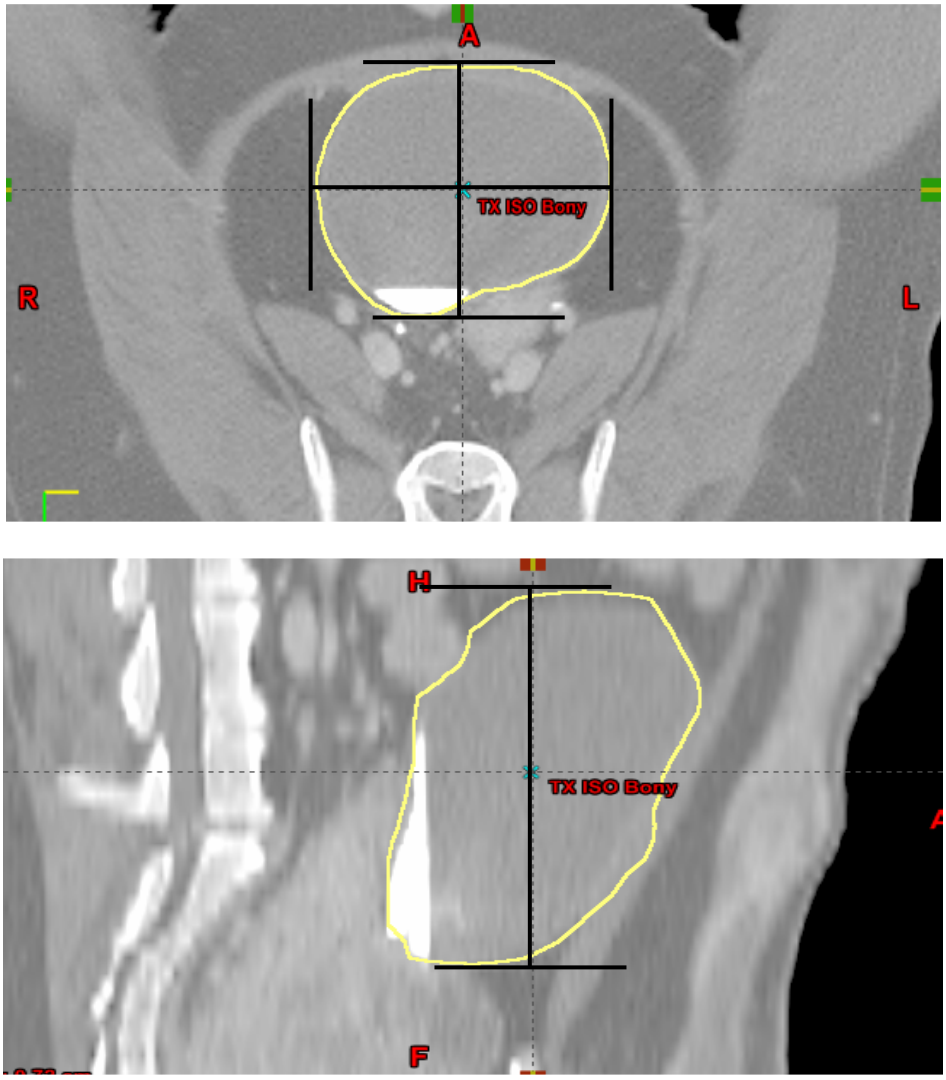


FIG. 2.2 Measurements were made from the center of mass of the bladder (TX ISO Bony) to the maximum bladder wall displacement in each of six directions. Axial view of patient showing how measurements were made in the right, left, dorsal and ventral directions (top). Sagittal view of patient showing how measurements were made in the cranial and caudal directions (bottom).

2.2e Parallel Opposed Treatment Plan Construction

Three different sample PTV structures were constructed for each dog by adding uniform 5 mm, 10 mm, or 15 mm margins to the original bladder volume structure, or the CTV, that was contoured on the planning CT (Fig. 2.3).

A standard plan was developed for each of the three PTV margins and applied to the images from each dog. The standard plan used for each dog consisted of two equally-weighted, conformal, right- and left-lateral parallel opposed fields that delivered 54 Gy in 20 fractions of 2.7 Gy with 10 MV photons. These plans were developed to theorize delivery based on location of anatomical structures in planning CT images. Plans were developed and analyzed as if they were to be delivered, however none of the plans were delivered clinically to the patients. Only computer-generated dosimetric models were analyzed. This dose scheme was chosen based on studies that showed administration of doses per fraction greater than 2.7 Gy increases the risk of late effects associated with the colon[11, 12]. Studies indicate that smaller doses per fraction may result in fewer or less severe acute effects with a subsequent reduction in consequential late effects[11].

Standard plans were prescribed to be delivered isocentrically with 100% of the prescription dose delivered to the geometric isocenter of the PTV. Field shaping, optimized for each PTV expansion, was achieved with multileaf collimators to simulate conventional blocking. The standard plans were improved using hard wedges, if needed, and a dose distribution was calculated with the

TPS (Fig. 2.4). The dose distribution for each of the three PTV expansion plans was then evaluated using DVH's that were calculated by the TPS.

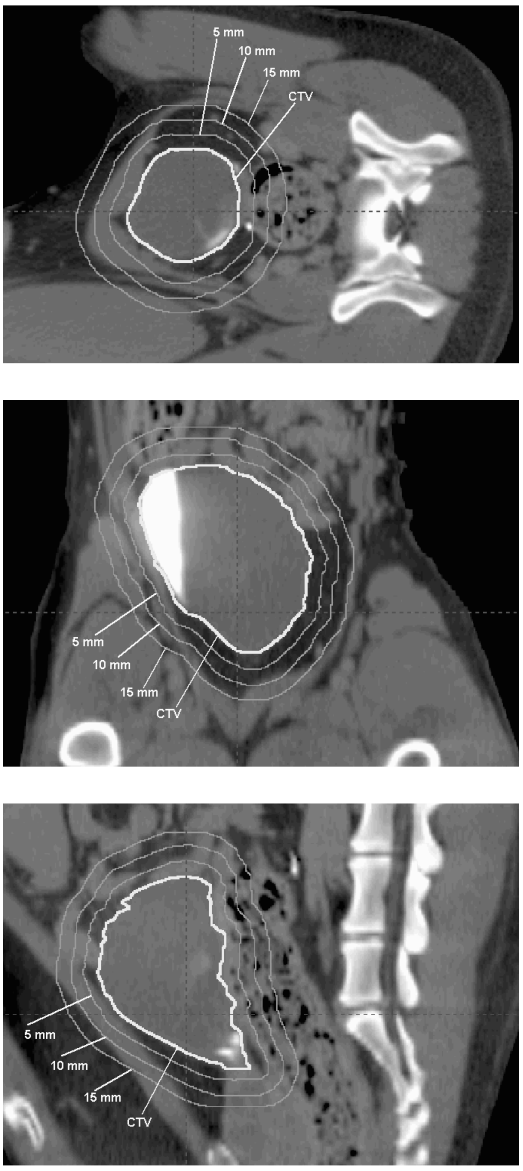


FIG. 2.3 Example of PTV expansions based on the planning CT bladder volume (CTV) for a dog in right lateral recumbency. Axial (top), coronal (middle), and sagittal (bottom) views.

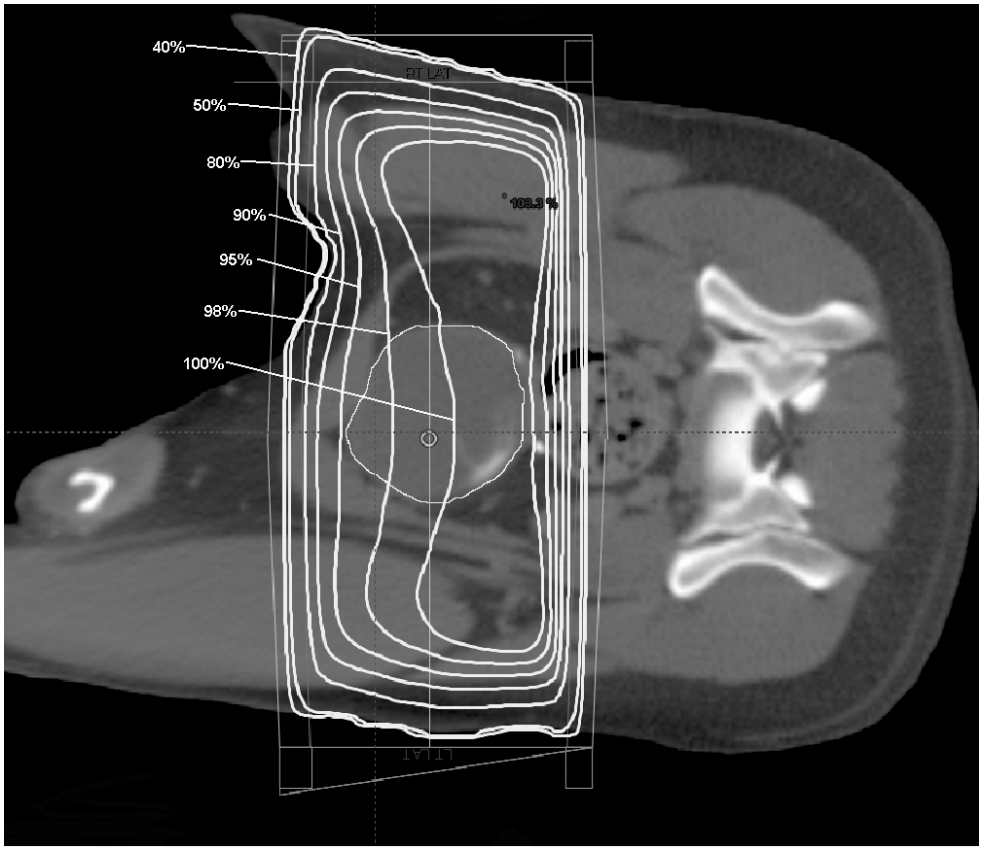


FIG. 2.4 Sample parallel opposed plan for dog in right lateral recumbency showing isodose lines and original bladder contour (CTV). The same plan was applied to each dog and dosimetric data was analyzed by DVH.

2.2f DVH Evaluation

The DVH showing the dose distribution of the PTV and the other organs of interest was calculated by the treatment planning system and was used to decide the acceptability of a treatment plan. DVH-derived information, like the dose to a given fractional volume, is often used as a measure of the quality of a dose distribution[58]. The ultimate goal of RT is to ensure that the target receives accurate and adequate dose coverage, while the dose to the critical structures is kept as low as possible[23].

2.2g Statistics

Variations in bladder wall position relative to planning CT bladder wall position in each of the six directions were compared between dogs in lateral, sternal and dorsal recumbency by examining the standard deviations. A mixed-model ANOVA was used with position as a fixed factor between dogs to investigate differences between positions in each of the six directions. Statistical significance was assumed at $P < 0.05$.

Mean changes in dose to the bladder and rectum were compared between dogs in lateral, sternal and dorsal recumbency. A mixed-model ANOVA was used with position as a fixed factor between dogs to investigate differences in dose between the three positions. Statistical significance was assumed at $P < 0.05$.

All statistical analysis was performed using SAS statistical software v. 10.0 (SAS, Chicago, IL, USA).

2.3 Results

2.3a Bladder Variations

The CBCT scans show a great amount of positional variation of the bladder on a daily basis in all three treatment positions. However, based on standard deviation (SD), the least amount of variation was seen in five out of the six directions for dogs in lateral recumbency. Dogs in lateral recumbency showed the least amount of bladder wall variation in the right (SD = 15.1%), left (SD = 20.0%), dorsal (SD = 20.5%), cranial (SD = 21.6%), and caudal (SD = 11.8%) directions when compared to the dorsally and sternally recumbent dogs. Table 2.1 summarizes the data. Bladder wall motion data are expressed as means, with ranges following in parentheses, ± standard deviation, for each of the six directions in each treatment position. Dogs in lateral recumbency showed a statistically significant difference in variability of the right bladder wall ($P = 0.04$) and of the caudal bladder wall ($P = 0.005$) when compared to dogs in dorsal or sternal recumbency. There was no statistical significance between treatment position and variability of bladder wall position in any other directions.

Based on the CBCT image data for dogs in dorsal recumbency, the mean percentage of right bladder wall variation was 100.6% (range, 46.6% - 161.7%) ± 24.0%; mean percentage of left bladder wall variation was 87.3% (range, 46.9% - 164.87%) ± 24.3%; mean percentage of dorsal bladder wall variation was 105.5% (range 65.5% - 175%) ± 23.3%; mean percentage of ventral bladder wall variation was 79.6% (range 20.9% - 158.1%) ± 24.4%; mean percentage of cranial bladder wall variation was 65.3% (range 0.1% - 145.45%) ± 32.1%; mean

percentage of caudal bladder wall variation was 98.5% (range, 58.3% - 131.8%) ± 14.0%.

For dogs in sternal recumbency, the mean percentage of right bladder wall variation was 90.7% (range, 38.7% - 121.4%) ± 15.7%; mean percentage of left bladder wall variation was 96.9% (range, 32.1% - 139.7%) ± 23.7%; mean percentage of dorsal bladder wall variation was 110.4% (range 38.7% - 144.2%) ± 25.6%; mean percentage of ventral bladder wall variation was 69.6% (range 3.0% - 113.3%) ± 33.1%; mean percentage of cranial bladder wall variation was 142.2% (range 50.0% - 316.7%) ± 82.2%; mean percentage of caudal bladder wall variation was 93.1% (range, 62.5% - 122.2%) ± 14.7%.

The CBCT image data for laterally recumbent dogs showed that the mean percentage of right bladder wall variation was 76.0% (range, 58.4% - 121.3%) ± 15.1%; mean percentage of left bladder wall variation was 101.0% (range, 50.8% - 140.8%) ± 20.0%; mean percentage of dorsal bladder wall variation was 91.7% (range 42.9% - 131.5%) ± 20.5%; mean percentage of ventral bladder wall variation was 105.8% (range 65.7% - 193.2%) ± 28.6%; mean percentage of cranial bladder wall variation was 83.4% (range 29.4% - 115.7%) ± 21.6%; mean percentage of caudal bladder wall variation was 81.2% (range, 50.0% - 105.8%) ± 11.8%.

Table 2.1 Average Percentage Variability in Bladder Wall Position on Daily CBCT Compared to Planning CT.

	Lateral[a,c]		*Dorsal*[a, d]		*Sternal*[a, e]	
Direction[b]	**Mean (Range)**	**Std Dev**	**Mean (Range)**	**Std Dev**	**Mean (Range)**	**Std Dev**
Right	76.0 (58.4-121.3)	15.1	100.6 (46.6-161.7)	24.0	90.7 (38.7-121.4)	15.7
Left	101.0 (50.8-140.8)	20.0	87.3 (46.9-164.9)	24.3	96.9 (32.1-139.7)	23.7
Dorsal	91.7 (42.9-131.5)	20.5	105.5 (65.5-175)	23.3	110.4 (38.7-144.2)	25.6
Ventral	105.8 (65.7-193.2)	28.6	79.6 (20.9-158.1)	24.4	69.6 (3.0-113.3)	33.1
Cranial	83.4 (29.4-115.7)	21.6	65.3 (0.1-145.45)	32.1	142.2 (50.0-316.7)	82.2
Caudal	81.2 (50.0-105.8)	11.8	98.5 (58.3-131.8)	14.0	93.1 (62.5-122.2)	14.7

a. Patient treatment positions.

b. Directions of bladder wall displacement relative to the common reference point.

c. Mean, range and SD calculated from data from 3 dogs, 29 total CBCT images (mean = 9 images per dog).

d. Mean, range and SD calculated from data from 3 dogs, 44 total CBCT images (mean = 15 images per dog).

e. Mean, range and SD calculated from data from 4 dogs, 37 total CBCT images (mean = 9 images per dog).

2.3b PTV Margins

The application of uniform 5, 10 and 15 mm margins to the planning CT bladder volume resulted in different dose distributions for each of the three treatment positions. Bladder dose was evaluated by using the DVH generated by the sample plan and determining the percentage volume of the bladder that received at least 95% of the prescribed dose. Adequate dose delivery to the bladder volume (CTV) is achievable in all three treatment positions, based on our criteria for this study (95% of prescribed dose to CTV). No statistically significant difference was found in bladder dose between the treatment positions.

Rectal dose was evaluated by determining the absolute volume (cc) of the rectum that received at least 100% of the prescribed dose. Mean dose to the rectum was lowest for dogs in lateral recumbency; however, there was no statistical difference in rectal dose found between the three treatment positions. Table 2.2 summarizes this data. Dose coverage of bladder and rectal volumes are expressed as means, with ranges following in parentheses ± standard deviation.

When the uniform five mm margin was applied to the bladder volume, dogs placed in dorsal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 91.1% (range, 54.6-98.7) ± 8.8% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 10.7 (range, 0.1-53.1) ± 11.3 cc. Dogs in sternal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 97.1% (range, 94.2-100.0) ± 2.0% with 100% of the prescription dose being delivered to

a mean rectal volume (cc) of 11.6 (range, 0.8-27.6) ± 7.4 cc. Dogs in lateral recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 93.0% (range, 81.3-99.8) ± 4.5% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 7.0 (range, 0.0-36.1) ± 7.9 cc.

For the uniform 10 mm margin, dogs placed in dorsal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 96.0% (range, 66.2-99.3) ± 6.2% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 14.2 (range, 0.4-63.9) ± 12.2 cc. Dogs in sternal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 98.0% (range, 95.9-100.0) ± 1.3% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 17.4 (range, 3.7-35.5) ± 9.3 cc. Dogs in lateral recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 98.4% (range, 87.0-99.9) ± 2.8% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 13.4 (range, 1.0-52.1) ± 9.8 cc.

When the 15 mm margin was applied, dogs placed in dorsal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 97.6% (range, 77.0-100.0) ± 4.2% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 28.1 (range, 1.0-79.1) ± 14.1 cc. Dogs in sternal recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 99.4% (range, 97.4-100.0) ± 0.7% with 100% of the prescription dose being delivered to a mean rectal volume (cc)

of 34.8 (range, 14.9-69.9) ± 13.2 cc. Dogs in lateral recumbency showed 95% of the prescription dose to cover a mean bladder volume percentage of 99.4% (range, 93.3-99.9) ± 1.2% with 100% of the prescription dose being delivered to a mean rectal volume (cc) of 22.4 (range, 5.0-71.0) ± 12.4 cc.

Table 2.2 Dose Coverage of Bladder (CTV) and Rectum.

	Lateral[a,d]		*Dorsal*[a, e]		*Sternal*[a, f]	
	Mean (Range)	**Std Dev**	**Mean (Range)**	**Std Dev**	**Mean (Range)**	**Std Dev**
Bladder (CTV) (%)[b]						
5 mm	93.0 (81.3-99.8)	4.5	91.9 (54.6-98.7)	8.8	97.1 (94.2-100.0)	2.0
10 mm	98.4 (87.0-99.9)	2.8	96.0 (66.2-99.3)	6.2	98.0 (95.9-100.0)	1.3
15 mm	99.4 (93.3-99.9)	1.2	97.6 (77.0-100.0)	4.2	99.4 (97.4-100.0)	0.7
Rectum (cc)[c]						
5 mm	7.0 (0.0-36.1)	7.9	10.7 (0.1-53.1)	11.3	11.6 (0.8-27.6)	7.4
10 mm	13.4 (1.0-52.1)	9.8	14.2 (0.4-63.9)	12.2	17.4 (3.7-35.5)	9.3
15 mm	22.4 (5.0-71.0)	12.4	28.1 (1.0-79.1)	14.1	34.8 (14.9-69.9)	13.2

a. Patient treatment positions.

b. Mean % of the CTV volume that received at least 95% of the prescribed dose.

c. Mean rectal volume, in cc, that received at least 100% of the prescribed dose.

d. Mean, range and SD calculated from data from 3 dogs, 29 total CBCT images (mean = 9 images per dog).

e. Mean, range and SD calculated from data from 3 dogs, 29 total CBCT images (mean = 9 images per dog).

f. Mean, range and SD calculated from data from 3 dogs, 29 total CBCT images (mean = 9 images per dog).

2.4 Discussion of Results

2.4a Characterization of Bladder Variations

A treatment plan is based on the pre-treatment imaging in most cases, with the treatment volumes being based on the pre-treatment bladder volume, location and shape. Unfortunately, these parameters are not always the same from day to day and such uncertainties can affect the treatment outcome. Based on the measurements from the daily CBCT data, we determined that the most optimal treatment position would be the one that would allow for the least amount of variation on a daily basis, which allows for the most reproducible treatment scenario each day.

Positioning in lateral recumbency allows for the least amount of bladder variation on a daily basis and results in the most reproducible treatment scenario each day. This was based on the smallest SD in five of the six directions for dogs in lateral recumbency, and was found to be statistically significant for the right ($P = 0.04$) and caudal ($P = 0.005$) bladder walls (Table 2.1).

These findings emphasize that, regardless of treatment position and efforts to standardize bladder volume through regular voiding, there can be substantial bladder wall movement from day to day. It is important for these values to be as consistent as possible because similarity of the daily bladder wall position to the original planning CT bladder wall position allows more accurate delivery of the dose to the bladder and minimizes dose to the surrounding tissues, resulting in fewer unacceptable complications. Although the bladder wall variations were not found to be statistically less for some of the directions measured, this may prove to be clinically significant if it enables treatment set-up

to be more reproducible from day to day when dogs are placed in lateral recumbency.

Minimal variation from the planning CT bladder wall position occurred in the caudal direction in all three treatment positions, with the lateral, dorsal and sternal positions all experiencing similar amounts of daily variation (SD = 11.8%, 14.0% and 14.7%, respectively) (Table 2.1) (Figs. 2.5A, B, C). This limited variation is most likely due to the fact that the caudal aspect of the bladder is continuous with the urethra and is limited in the amount of motion it can experience.

Human studies have found bladder motion to be most pronounced in the cranial and anterior directions[8, 47], and in this study of canine bladder wall variations, motion was found to be most pronounced in the cranial and ventral directions. There was a large amount of variation in the daily bladder wall position from the planning CT bladder wall position in the cranial direction for dogs in sternal recumbency (142.2% ± 82.2%) (Fig. 2.5). We have attributed this to the fact that, when the dogs are placed in this position, the treatment couch limits the variation in the ventral direction, the spine limits the variation in the dorsal direction and the urethra limits the variation in the caudal direction, so the bladder wall position varies in the cranial direction with daily changes in volume. We hypothesize that this may also be the reason that dogs in lateral recumbency show a significantly smaller amount of variation in bladder wall position in the right direction and show the greatest amount of variation in the ventral direction. The right bladder wall would be limited in its motion by the

treatment couch. The larger amount of variation observed by the ventral wall would not be limited by physical restriction and could freely change position as the position or volume of the bladder changed.

Of the six directions, laterally recumbent dogs showed the largest SD, and largest amount of variation in the ventral direction (Table 2.1) (Fig. 2.5A). When dogs are in lateral recumbency, the ventral bladder wall has no physical restriction to motion and, like the cranial direction, the bladder moves freely in this direction as it fills. The ventral direction was the direction with the second-highest amount of bladder wall variation for dogs in dorsal recumbency (79.6% ± 48.8%) (Fig. 2.5B) and dogs in sternal recumbency (69.6% ± 66.2%) (Fig. 2.5C).

A solution to compensate for the pronounced bladder wall motion in the cranial and ventral directions would be to asymmetrically increase the PTV margin in the cranial and anterior directions. Another option would be to use adaptive RT to account for these shape changes for each individual patient on each individual day.

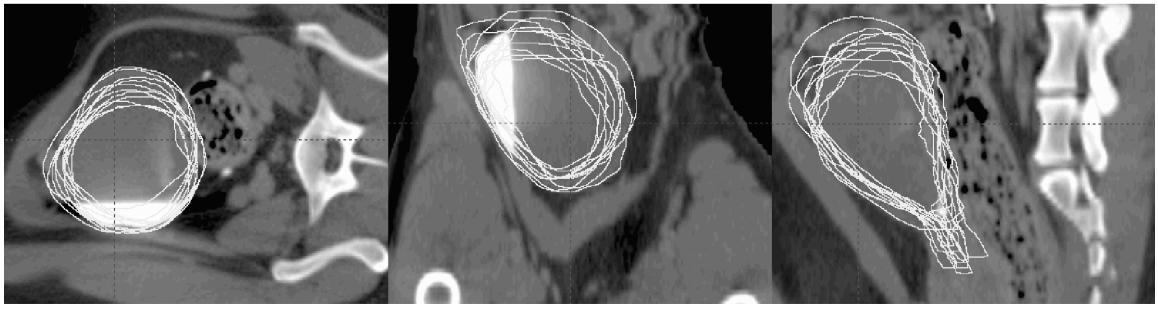


Fig. 2.5A Bladder wall variations for dog in right lateral recumbency. Dogs in lateral recumbency showed the greatest amount of bladder wall variation ventrally and cranially. Lateral recumbency allows for the least amount of variation in bladder wall position on a daily basis.

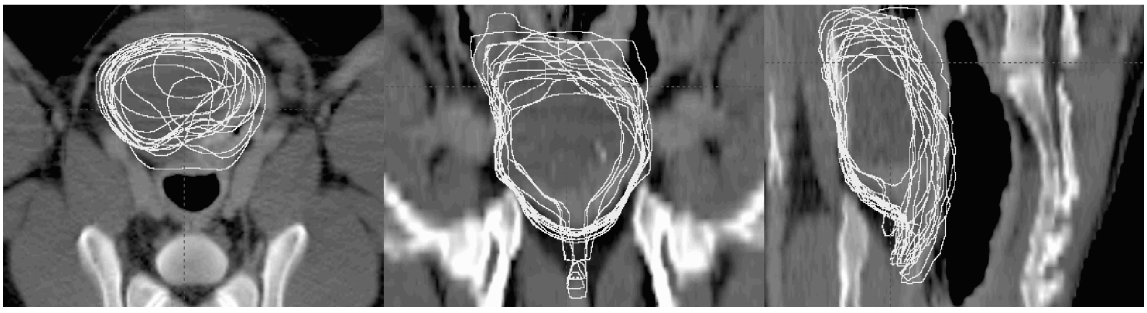


Fig. 2.5B Bladder wall variations for dog in dorsal recumbency. Dogs in dorsal recumbency showed the greatest amount of bladder wall variation in cranially and ventrally.

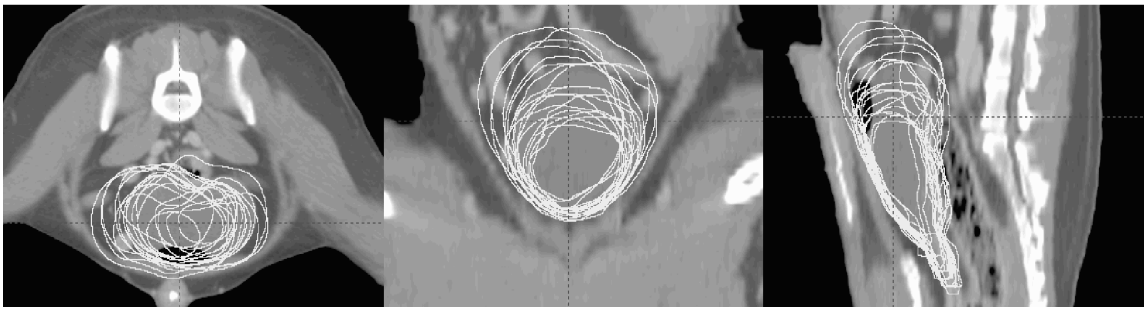


Fig. 2.5C Bladder wall variations for dog in sternal recumbency. Cranial variations were largest in this position due to the restricted bladder wall motion in five of the six directions.

2.4b Evaluation of Patient Position and PTV Expansions

Dose volume histograms were used to analyze the dosimetric data for each PTV expansion in each treatment position (Figs. 2.6, 2.7, 2.8). Dogs in lateral recumbency showed the least amount of variation in bladder wall position and subsequently showed the best dose distribution for each of the three PTV expansions using parallel opposed RT plans. This information will prove to be helpful in choosing the best treatment set-up scenario for bladder cancer patients at all veterinary radiation therapy facilities.

Based on the practices common in our clinic we chose to assess the percent volume of the bladder receiving 95% of the 54 Gy prescription dose and, as expected, found each increasing PTV expansion to provide increasing bladder coverage for all three treatment positions (Table 2.2). No statistical differences were found between treatment positions for dose coverage to the bladder CTV.

The goal was to determine the PTV expansion that would maximize the percentage of the bladder volume receiving 95% of the prescription dose while simultaneously minimizing the rectal volume that would receive 100% of the prescription dose. Studies in humans receiving abdominal or pelvic irradiation are conflicting in their conclusions about volume effects and late gastrointestinal radiation, and the criteria used to define large versus small volumes differs substantially in these studies. A similar study in rats was not able to show a correlation of field size or length of colon in the field to severity of late effects[59]. However, in a study of pelvic irradiation in dogs, the entire rectal circumference was irradiated and the dogs receiving higher doses showed more severe late

effects[11]. Thus, we decided to examine the rectal volume receiving the total dose. The lateral treatment position best minimized dose to the rectum using the 5, 10 and 15 mm expansions, with only 1.9, 5.4 and 16.1 cc, respectively, receiving the full prescription dose. Limiting the dose to the rectum via lateral positioning may prove to be clinically significant, although not statistically significant, as previous studies have found a correlation between fraction size and adverse effects[11], as well as support using more conformal techniques in order to reduce dose to sensitive normal tissues and reduce complication rate[12].

Although each clinic will have unique and specific planning goals with respect to maximum rectal dose allowed and bladder coverage critera, an ideal expansion provides a good compromise between adequate bladder coverage while minimizing rectal dose.

PTV optimization was performed by simultaneously plotting the average percentage of the bladder volume that received less than 95% of the 54 Gy prescription dose and the average rectal volume (cc) that received the 54 Gy prescription dose or greater for each of the PTV expansions. One graph was produced for each of the three treatment positions (Figs. 2.9, 2.10, 2.11). Optimal expansion would be the intersection of the two lines in each plot. The 10 mm expansion offers the best compromise of bladder coverage and minimal rectal volume irradiated for all three treatment positions.

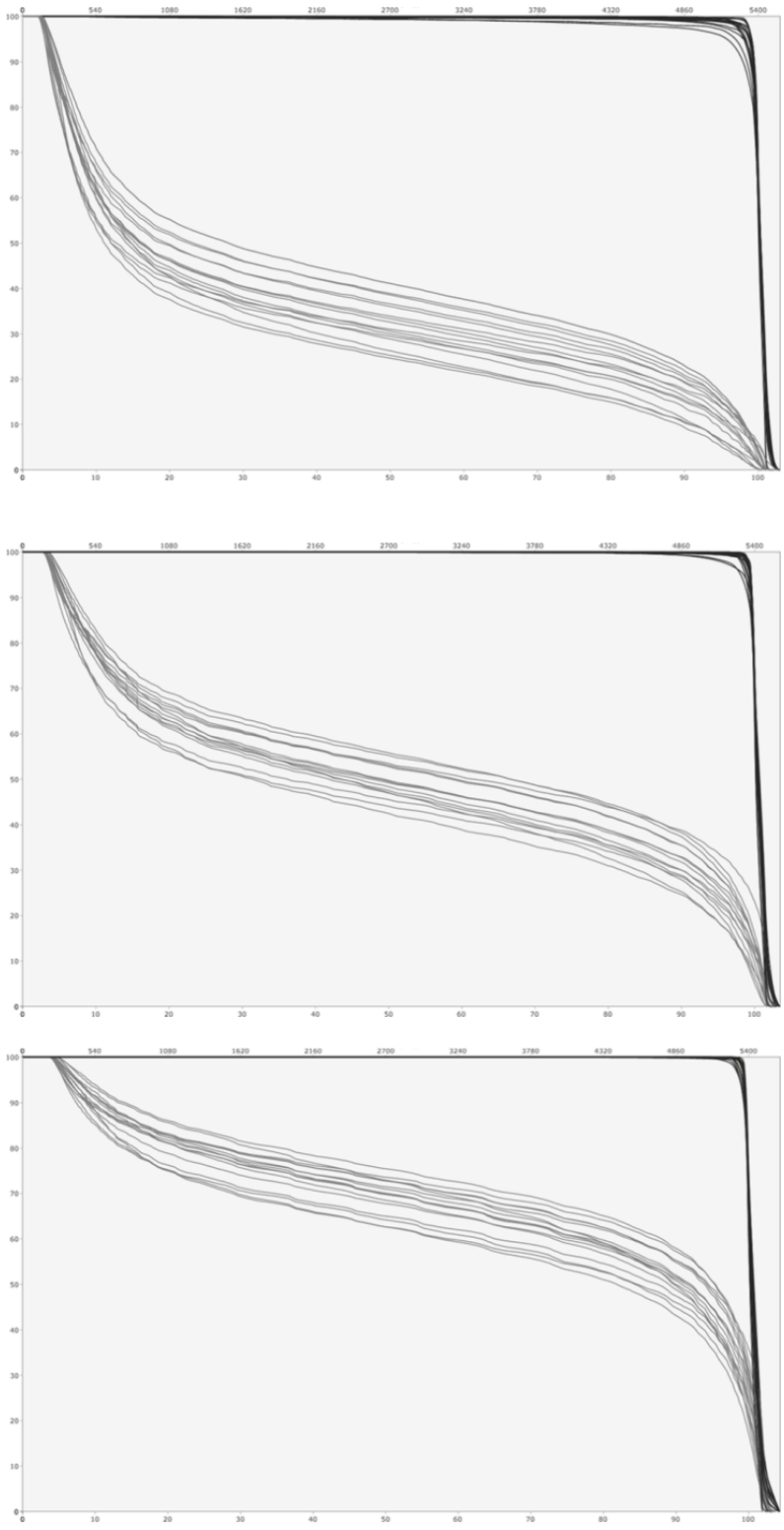


Fig. 2.6 Lateral position dose volume histograms for 5(top), 10(middle) and 15(bottom) mm PTV expansions. Bladder dose (black) and rectal dose (gray).

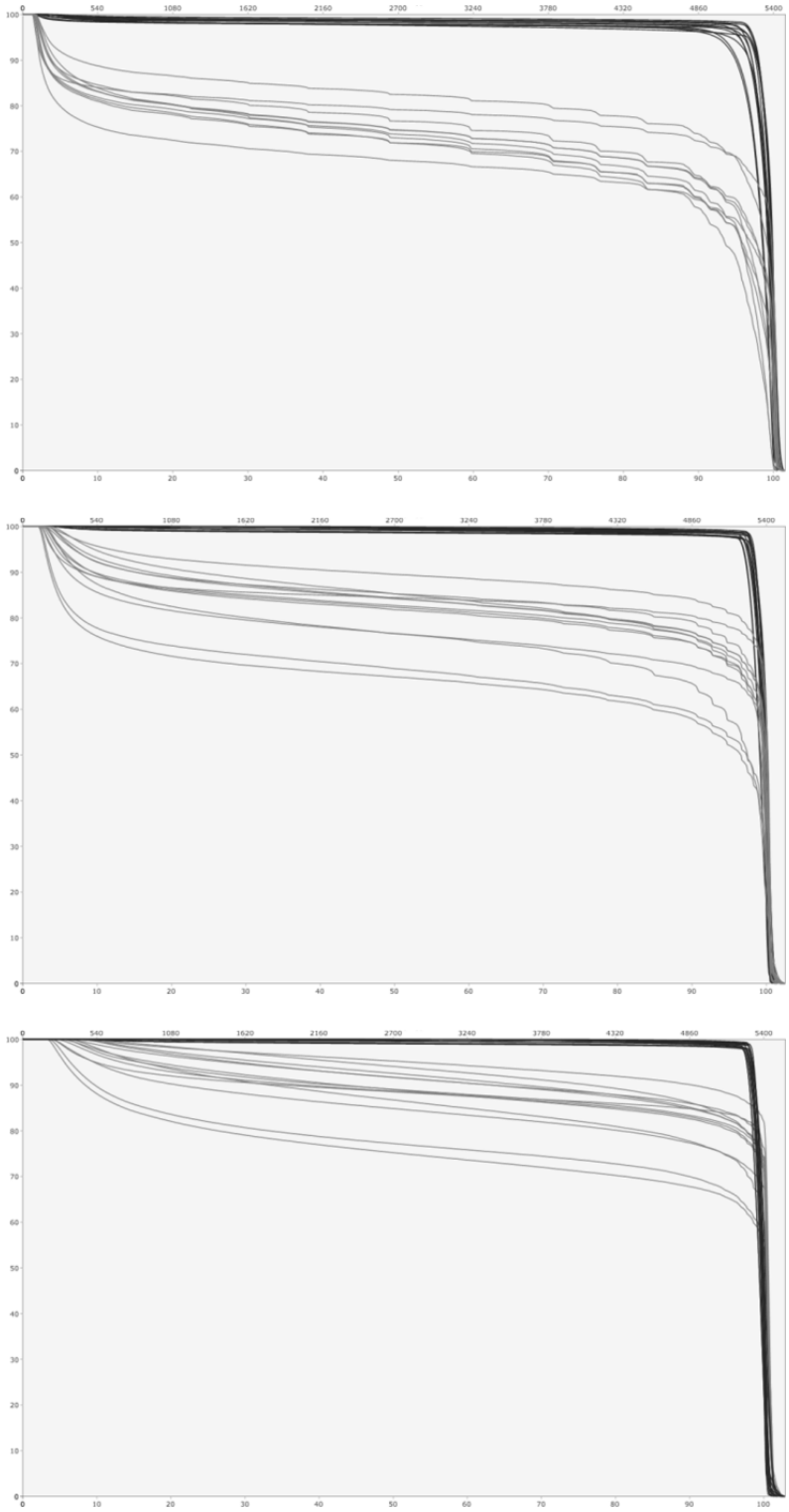


Fig. 2.7 Dorsal position dose volume histograms for 5(top), 10(middle) and 15(bottom) mm PTV expansions. Bladder dose (black) and rectum dose (gray).

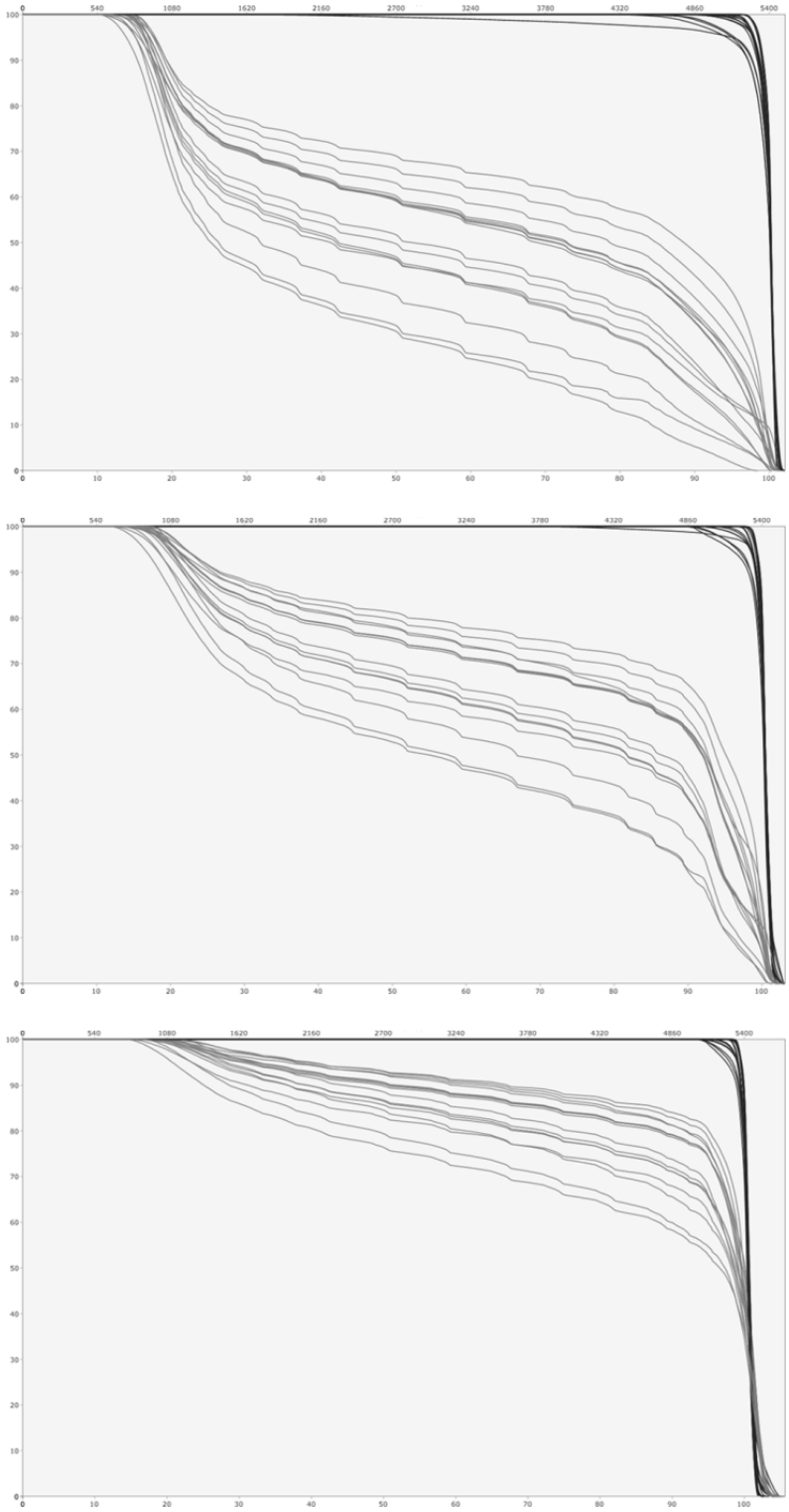


Fig. 2.8 Sternal position dose volume histograms for 5(a), 10(b) and 15(c) mm PTV expansions. Bladder dose (black) and rectum dose (gray).

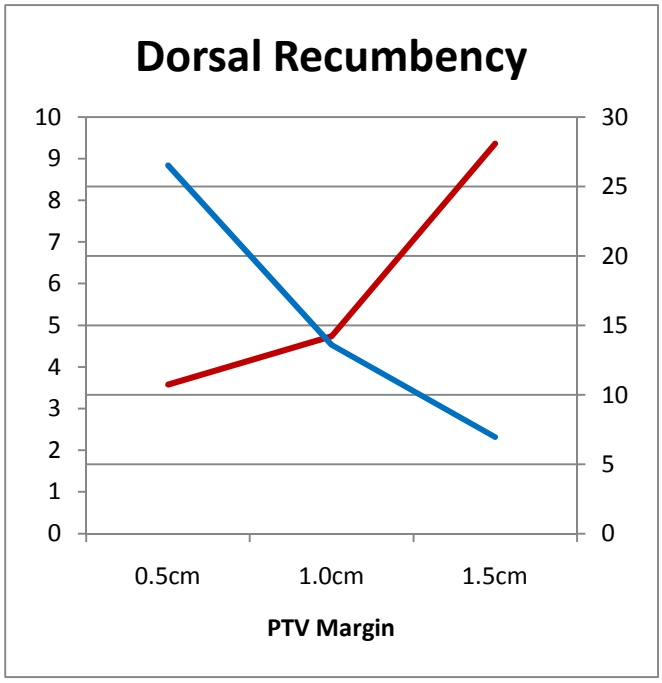


Fig. 2.9 Graph for dogs in dorsal recumbency showing average percentage of bladder volume that receives less than 95% of 54 Gy prescription dose (left axis, blue line) and average rectal volume (cc) receiving 54 Gy prescription dose or greater (right axis, red line) for 5, 10 and 15 mm PTV expansions. Optimal margin is at intersection of plots, or 10 mm.

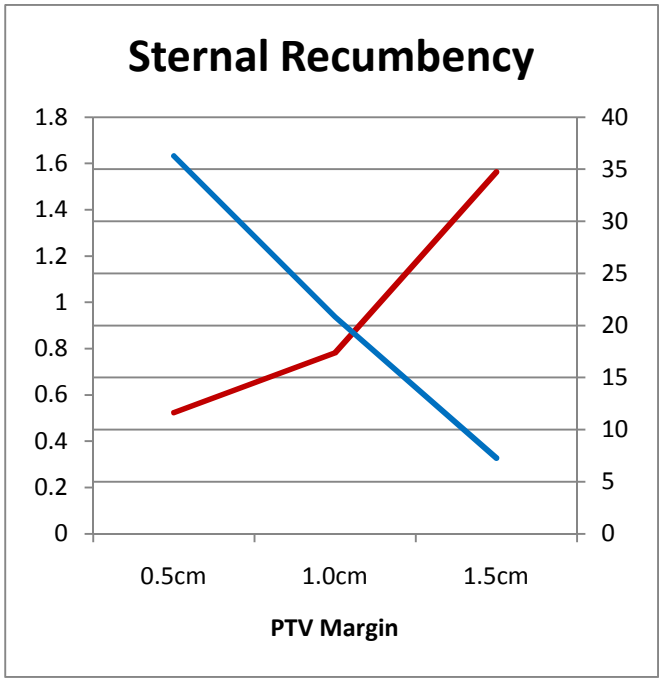


Fig. 2.10 Graph for dogs in sternal recumbency showing average percentage of bladder volume that receives less than 95% of 54 Gy prescription dose (left axis, blue line) and average rectal volume (cc) receiving 54 Gy prescription dose or greater (right axis, red line) for 5, 10 and 15 mm PTV expansions. Optimal margin is at intersection of plots, or 10 mm.

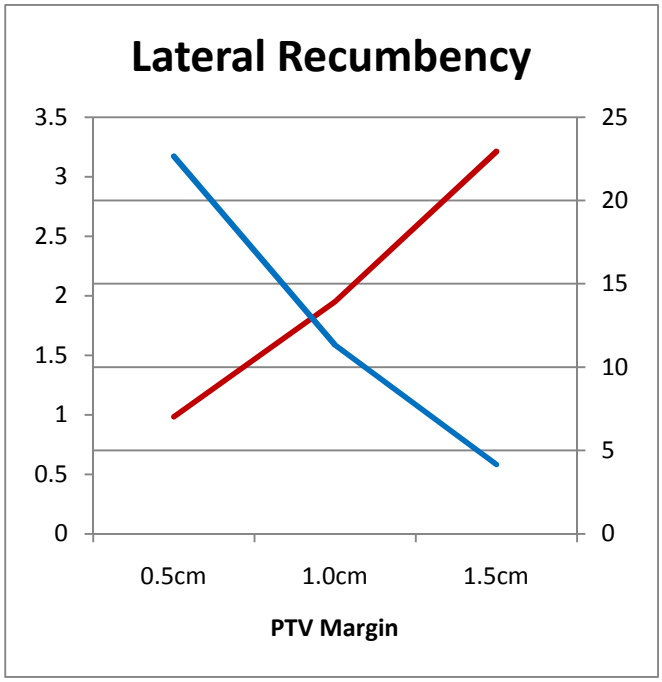


Fig. 2.11 Graph for dogs in lateral recumbency showing average percentage of bladder volume that receives less than 95% of 54 Gy prescription dose (left axis, blue line) and average rectal volume (cc) receiving 54 Gy prescription dose or greater (right axis, red line) for 5, 10 and 15 mm PTV expansions. Optimal margin is at intersection of plots, or 10 mm.

The overall dose distribution seen on the DVH's for the lateral dogs is optimal of the three positions (Fig. 2.6). In addition to the lateral treatment position experiencing the smallest amount of overall bladder wall variation, another possible explanation for this was noticed while constructing the contours on the daily CBCT images. The bladders of dogs in dorsal or sternal recumbency, as noted in the above section, are restricted in movement in certain directions due to the treatment couch or another anatomical structure, the most noticeable of these being the rectum. Despite efforts to produce the same bladder and rectal volumes from day to day, there are days when the rectal volume is larger than the rectal volume of the planning CT. This causes the rectum to displace the bladder where they are closest and cause a deformation in the bladder that differs from the bladder shape on the original planning CT (Fig. 2.12). A substantial portion of the rectum then occupies the same space that was previously designated in the treatment plan as the bladder. Since the planning CT volumes are used to produce the RT plan, the rectum is directly irradiated on the days when it displaces the bladder. This phenomenon was not noticed with the dogs positioned in lateral recumbency, as there is less variation in the motion of the rectum and the bladder is able to move away from the rectum, often producing physical separation between the two structures, thus reducing the rectal volume that is irradiated.

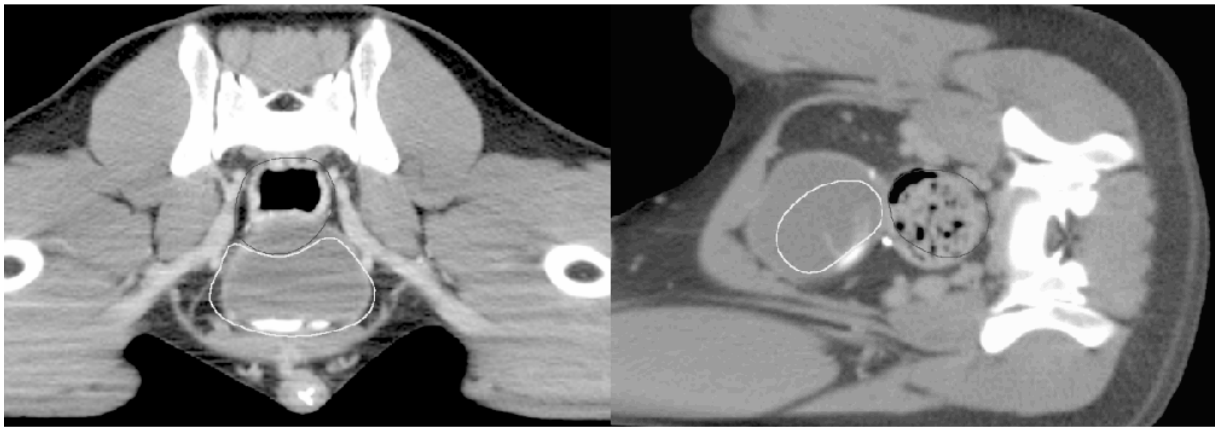


FIG. 2.12 Bladder deformation experienced by dogs in sternal or dorsal recumbency is not noticed in dogs in lateral recumbency. (a) CBCT data fused onto the planning CT shows that large rectal volumes can deform the bladder and cause the daily bladder shape to differ from the original planned shape that will be irradiated. (b) Lateral CBCT data fused onto planning CT does not show this phenomenon, as the bladder and rectum are able to move apart in this position.

CHAPTER 3
IMRT, IGRT and ART

3.1 Introduction to Advanced Techniques in Radiation Therapy

Techniques such as 3D conformal RT (CRT) and intensity modulated RT (IMRT) were developed to improve upon traditional, non-CT based treatment setups with large radiation fields, uniform-intensity beams, and a high probability of radiation-related side effects. Advanced RT techniques are based on 3D anatomic information and use dose distributions that conform as closely as possible to the target volume (Fig. 3.1). Conformality allows for a higher dose to be delivered and minimizes the probability of normal tissue complication. Conformal radiotherapy offers the greatest advantage at sites, such as the bladder, where existing local control is limited by the collateral dose to nearby normal structures[5], such as the rectum.

Despite the advantages of using a conformal RT technique, there are some potential obstacles. Accurate assessment, localization and delivery are vital to improve tumor control and reduce normal tissue toxicity in conformal radiotherapy[60]. Organ motion and setup errors provide possibilities for the tumor target to vary in position each day, and must be accounted for with an adequate PTV margin. In addition, the higher doses that are often used with these types of therapies need to be accurately targeted and delivered to the tumor each day to avoid irradiation of nearby normal tissues.

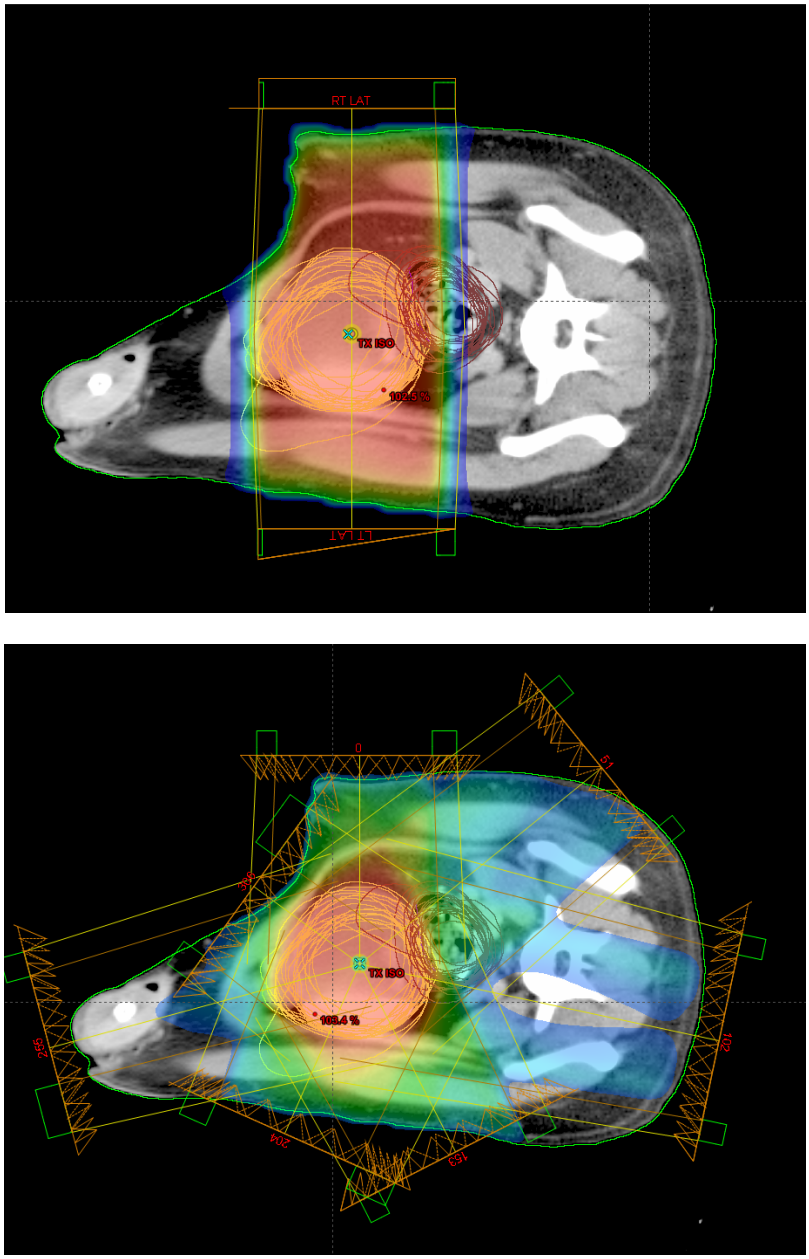


Fig. 3.1 Colorwash dose distribution for (top) parallel opposed, and (bottom) IMRT treatment plans. IMRT delivers a more conformal dose to the bladder target with steep dose gradients leading out towards nearby normal tissue.

3.1a Intensity Modulated Radiation Therapy (IMRT)

Intensity modulation is the process of modulating the beam intensity profiles so that a specific planning goal is met. This can be accomplished with wedges or, in the case of IMRT, multi-leaf collimators (MLC). IMRT is an advanced form of CRT that delivers non-uniform fluence to the target from several different beams with the goal being delivery of a composite, conformal dose distribution. Each beam is weighted so as to achieve the final dose distribution, as predetermined by the treatment planning system (TPS). The TPS determines the optimum intensity modulation, as decided through inverse planning, and limits the dose to the surrounding normal tissue.

The computer-controlled MLC shape the field in a static or dynamic delivery, or some combination of the two, to achieve the conformal dose in IMRT. In the static delivery method, the leaves move to shape each sub-field, the beam is turned on and then switched off while the leaves position themselves into the next sub-field position. This is also sometimes known as step-and-shoot delivery. With the dynamic delivery method, the leaves simultaneously sweep from one side to the other and the radiation is delivered as the leaves are moving. This method is sometimes known as the sliding window method. The gantry rotates to the next position and the next beam is delivered. This process is repeated until all beams have been delivered and the total fractional dose has been delivered.

IMRT delivery has provided an effective way to shape dose distributions to fit complex tumors while concurrently sparing normal tissues close to the target

volume. Dose conformality is essential to escalate the radiation dose in order to improve the outcome of bladder RT and avoid increased normal tissue adverse effects[52]. IMRT is superior to CRT with respect to sparing of the bowel[61]. In human studies, IMRT has demonstrated a reduction in both acute and late radiation morbidity compared to conformal techniques[21, 23].

Despite the success rates seen in human medicine, IMRT is still a relatively uncommon practice in veterinary medicine, especially for the treatment of canine bladder cancer. A very modern, late model linear accelerator or tomotherapy unit is needed to deliver IMRT and is not readily available to all veterinary facilities. Another complicating factor is the need for a better understanding of the motion characteristics of the canine pelvic organs in order to precisely deliver the higher radiation doses used with IMRT and to avoid delivering these high doses to nearby critical structures. If these motion characteristics are better understood, it may be possible to achieve a success rate similar to that seen in human medicine using IMRT for canine bladder cancer patients.

3.1b Image Guided Radiation Therapy (IGRT)

A fundamental principle of RT is that a successful treatment outcome requires accurate alignment of the treatment field to the target tissue volume[5], especially for highly-conformal methods such as IMRT, which produce such steep dose gradients outside of the PTV. Therefore, adequate visualization of the tumor target and its surrounding organs is a critical step in the process of

improving RT. However, during the course of radiotherapy, target volumes can change position due to motion from surrounding internal organs and physiological processes. The conformal dose distributions and steep dose gradients generated around target volumes when using IMRT require an accurate, reproducible treatment setup with monitoring and verification at the time of treatment set-up to prevent a geographic miss or over-irradiation of nearby normal tissues. Accuracy in localization becomes even more important with the delivery of high doses each day using IMRT [62]. It therefore seems advantageous to use image guidance to safely deliver such treatments.

Image guided RT (IGRT) is the use of imaging technology to assist in the delivery of RT, such as IMRT, to the appropriate target volume. Different types of imaging can be used, such as kV orthogonal, MV portal or CBCT. The images are acquired after the patient has been placed in the daily treatment position and immediately prior to the delivery of that daily RT fraction. The daily images are then used to make position adjustments to properly align the patient anatomy to the linac isocenter. The planned dose is not changed from day to day, however minor position adjustments enable the daily target volume to be better aligned to match the treatment plan than if image guidance was not utilized. The images help to more accurately localize the target volume and avoid irradiating the surrounding tissues. Increased accuracy in tumor localization also allows for a decrease in the PTV margins due to decreased uncertainty regarding the interfraction motion and setup errors that can occur on a daily basis[21]. This

decreased PTV margin lowers the total volume irradiated and the possibility of radiation-induced side effects.

Volumetric imaging of the area of interest is becoming an integral part of IGRT, with many studies reporting significant improvements in the visibility of anatomic structures, soft tissues in particular, compared with megavoltage portal imaging[63]. 3D imaging systems, such as cone beam computed tomography (CBCT), can improve daily setup and therefore increase the accuracy of RT delivery. Kilovoltage (kV) CBCT can be mounted on the linear accelerator gantry and permit the volumetric position verification of the tumor volume and surrounding organs at risk, relative to the treatment geometry, immediately prior to treatment. Thus, CBCT systems allow online correction of patient setup errors, immediately prior to initiation of RT[64]. CBCT allows the treatment of tumors that were not easily or effectively treated before by enabling accurate patient setup.

3.1c Adaptive Radiation Therapy (ART)

The emergence of IGRT and newer imaging technologies has made the improvement of many types of RT possible by allowing visualization of the tumor immediately prior to treatment while the patient is in the treatment position and allowing for adjustments to better target the tumor volume. However, some tumors, such as bladder tumors, require the treatment of volumes that not only change position, but can change shape and volume as well. A standard RT protocol can only account for position changes because it employs the same plan

each day that was based on the original planning CT volumes and their positions. If the daily target volume lies outside of the original PTV, there is the risk for geographic miss and lowered tumor control. If the daily target volume is much smaller than the original treatment volume, there is the risk for irradiation of the normal tissue that is nearby and an increase in probability of subsequent radiation-induced side effects. An adaptive radiotherapy (ART) protocol that reviews daily images in order to implement changes and adapt the treatment plan before delivery will facilitate visualization and assessment of both setup and random daily organ movement and volume variations.

ART, sometimes referred to as dynamic adaptive RT (DART), is a technique that re-optimizes RT throughout the course of the treatment on an individual patient basis[53]. It uses daily images, acquired at the time of each treatment, to develop and deliver a new RT plan each day, based on that day's anatomy. ART can occur daily (online), during treatment (real time), or it can occur between fractions (offline). Offline adaptation takes place between treatment fractions based on new information that can be used to adapt treatments for gradual changes in patient or tumor anatomy, physiology, or setup. Real-time adaptation involves techniques such as respiratory gating to gate the beam during treatment to account for internal organ motion, such as lung motion during the breathing cycle. Treatment machine parameters are adapted in real time to conform to the patient anatomy in real time. Online adaptation updates the treatment parameters based on new daily information to account for variations in patient anatomy or physiology for which repositioning

the patient alone cannot correct. Online adaptation ensures that the treatment objectives are continuously met despite changes in the patient[5] or position of the target.

ART is superior to traditional CT-based treatment planning because traditional planning utilizes a single image data set of the patient, which may not be an accurate representation of anatomic shape and position on each day of a prolonged course of treatment. Uncertainties are typically incorporated into the PTV margins when a single CT data set is used for planning and result in a larger irradiated volume. The ART strategy leads to a substantial reduction in treatment volumes and improved targeting of those volumes when compared with conventional strategies in human studies[6]. The ART approach can reduce toxicity and allows for the possibility of dose escalation, which could lead to improvement of RT treatment outcomes. ART could be especially useful for the treatment of canine bladder cancers, as the tissue volume and location can vary from day to day.

3.2 Materials and Methods

3.2a Patient Selection

The bladder motion and PTV expansion data from the first aim of this study showed that dogs placed in lateral recumbency had the most reproducible setup and best dose distribution on a daily basis. Thus, image data used to construct the following treatment plans were from four dogs positioned in lateral recumbency. The four dogs were treated with standard-of-care IMRT and

received daily CBCT scans for positioning purposes. Twenty CBCT data sets were available for each patient, for a total of 80 CBCT data sets. Each type of plan was constructed using the data sets from all four dogs.

3.2b Bony Anatomy Registration

Owing to the fact that many human studies have found that IMRT reduces both acute and late radiation morbidity compared to conformal techniques[21, 23], the possibility for duplicating human results in canine bladder cancer patients was explored. Daily CBCT images were registered to the planning CT for each of the four laterally recumbent dogs using a bony anatomy registration technique (Section 2.2c) and dose distributions evaluated for each daily bladder volume. The daily bladder and rectal contours were copied from each daily CBCT image to the original planning CT image which shared the same bony anatomy frame of reference.

IMRT plans for each of the four dogs were then constructed by adding a uniform 5 mm PTV margin to the original planning CT bladder volume. This smaller margin was examined for this technique because it was hypothesized that the more conformal techniques would allow for a reduction in the PTV volume in order to spare the nearby healthy tissues. A typical 7-field IMRT technique (0, 51, 102, 153, 204, 255, and 306-degree field gantry angles) was used with the field intensity modulated by the MLC during sliding window delivery. The TPS uses an iterative optimization to focus dose on the PTV and avoid the rectal volume. The treatment dose (54 Gy in 20 fractions of 2.7 Gy)

was isocentrically prescribed to the PTV using 6 MV photons. Dosimetric data was generated by the TPS and displayed on the DVH for plan review. The dose distribution for each daily bladder and rectal volume was evaluated by DVH.

3.2c Soft Tissue Registration

A different type of registration based on soft tissues can be used to improve the delivery of RT to tissues that experience a wide range of motion and shape changes. The CBCT images, after being transferred to the TPS, were registered to the planning CT image set. Registration was accomplished using a soft tissue registration. This registration technique does not use bony landmarks as reference points, but rather matches soft tissue organs of interest. For the purposes of this study, the bladder structure from each daily CBCT was matched, as close as possible, to the original bladder structure from the planning CT. The bladder volumes contoured on the CBCT scans were copied to the planning CT so they would be in the same soft tissue frame of reference, based on the bladder position for each day.

Soft tissue image guided registration takes organ motion into account for the subsequent treatment delivery and allows for a more accurate fit of the daily bladder volume to the original PTV (Fig. 3.2) by allowing the bladder to be "chased" using CBCT images. Accuracy of treatment delivery is crucial to effective RT, and especially when delivering high radiation doses with techniques such as IMRT.

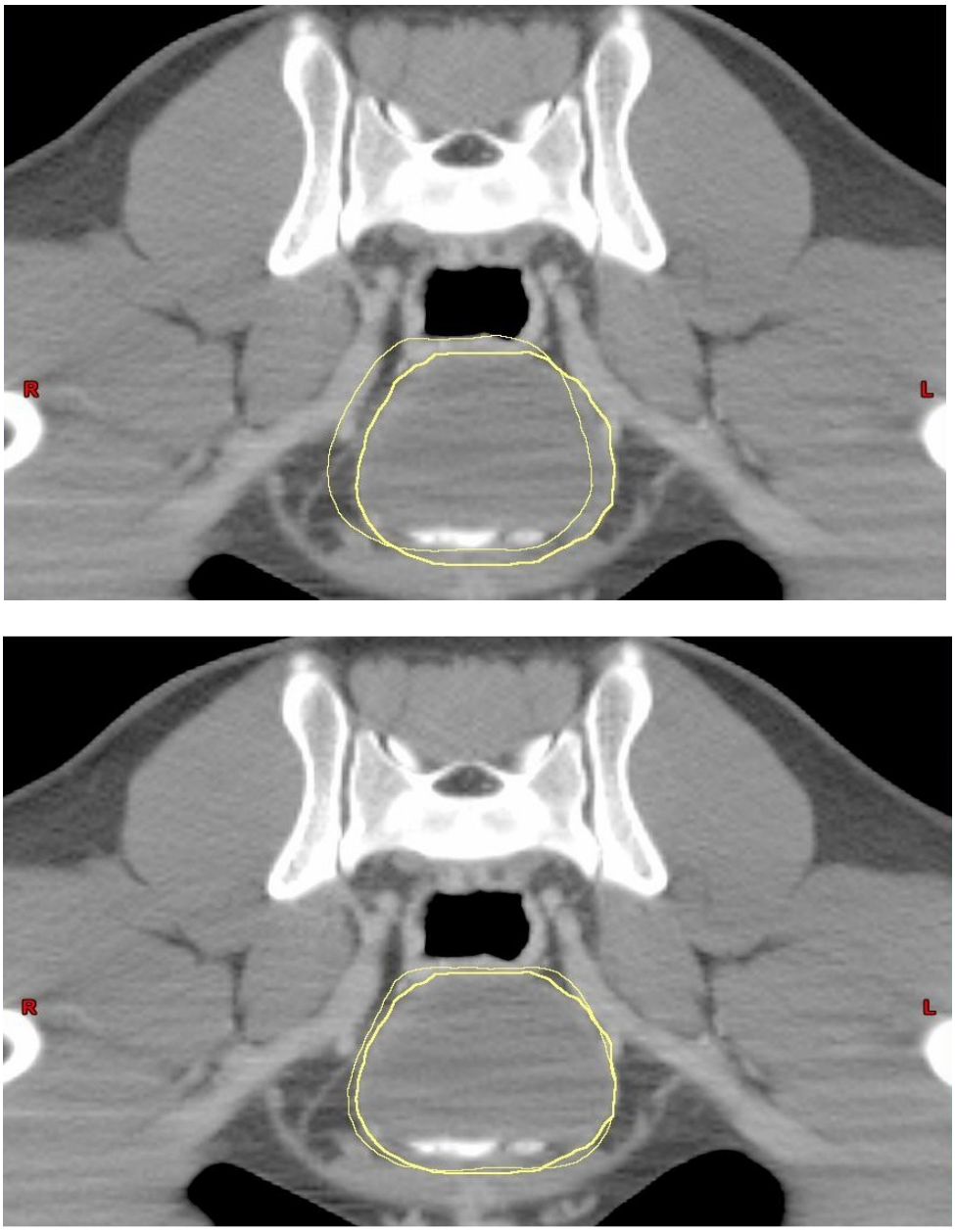


FIG. 3.2 Planning CT with daily CBCT bladder volume copied after registration. Bony anatomy registration (top) positions patient based on bony structures, but does not take into account organ motion and produces a discrepancy in bladder volume positions. Soft tissue registration (bottom) is based on the bladder itself and provides better targeting of the bladder volume each day.

3.2d Soft Tissue Registration Plan Construction

The use of IMRT to deliver high doses with steep dose gradients requires accurate localization of the daily target and delivery of the radiation[62]. The ability to use CBCT for localization of the bladder and soft tissue registration to target the IMRT dose makes curative IGRT for canine bladder cancer a possibility.

The CBCT data sets for each of the four dogs in lateral recumbency were registered to the planning CT using a soft tissue registration method, in order to evaluate the dose distributions for the daily bladder volumes. The daily bladder and rectal contours were copied to the planning CT and now utilized the same soft tissue frame of reference.

The IGRT treatment plans for each of the four dogs were then constructed using the same 5 mm PTV expansion and 7-field IMRT technique as used for the bony anatomy registration, IMRT plans (Section 3.2b). Again, the DVH displayed the dosimetric data generated by the TPS and was used for evaluation of the IGRT plans.

3.2e ART Plan Construction

IGRT with CBCT provides the ability to clearly image the internal anatomy each day prior to treatment and to target the correct volume. The radiation treatment plan could then be modified each day based on the imaging information and the development of an ART plan to treat canine bladder cancer.

The daily CBCT image data sets were transferred to the TPS and registered to the planning CT using a bony anatomy registration technique

(Section 2.2c). A bony anatomy registration was chosen for its simplicity and to minimize time requirements, as compared to a soft tissue registration. The daily bladder and rectal contours were then copied from each daily CBCT image to the original planning CT image using the same bony anatomy frame of reference. The bladder contours included the entire volume encompassed by the bladder wall, including the bladder contents. The transverse colon was the cranial boundary and the caudal boundary was 2 cm past the caudal end of the urinary bladder to include the trigone area and urethra. The length of the rectum adjacent to the bladder was also contoured and included the entire rectal volume and its contents encompassed by the outer rectal wall. All organ contours were drawn on each slice by hand by the same investigator (JRN) to ensure consistency.

A new ART treatment plan was generated based on each daily bladder volume for each of the four dogs. Using CBCT allowed for improved bladder localization and we chose a slightly smaller PTV expansion. A 3 mm PTV margin was added to each contoured bladder volume copied to the planning CT and was used as the new daily PTV for each new treatment (Fig. 3.3). Again, this smaller PTV margin was chosen due to the increased conformality of this technique and the ability to better target the tumor volume. A new 7-field IMRT plan was developed based on each daily target volume. The TPS uses an iterative optimization to focus dose to each daily PTV and avoid each daily rectal volume. The treatment dose (54 Gy in 20 fractions of 2.7 Gy) was isocentrically prescribed to the daily PTV using 6 MV photons in 20 individual daily plans over

the course of the treatment. The dose distribution for each daily plan was evaluated using the dosimetric data from the TPS-generated DVH.

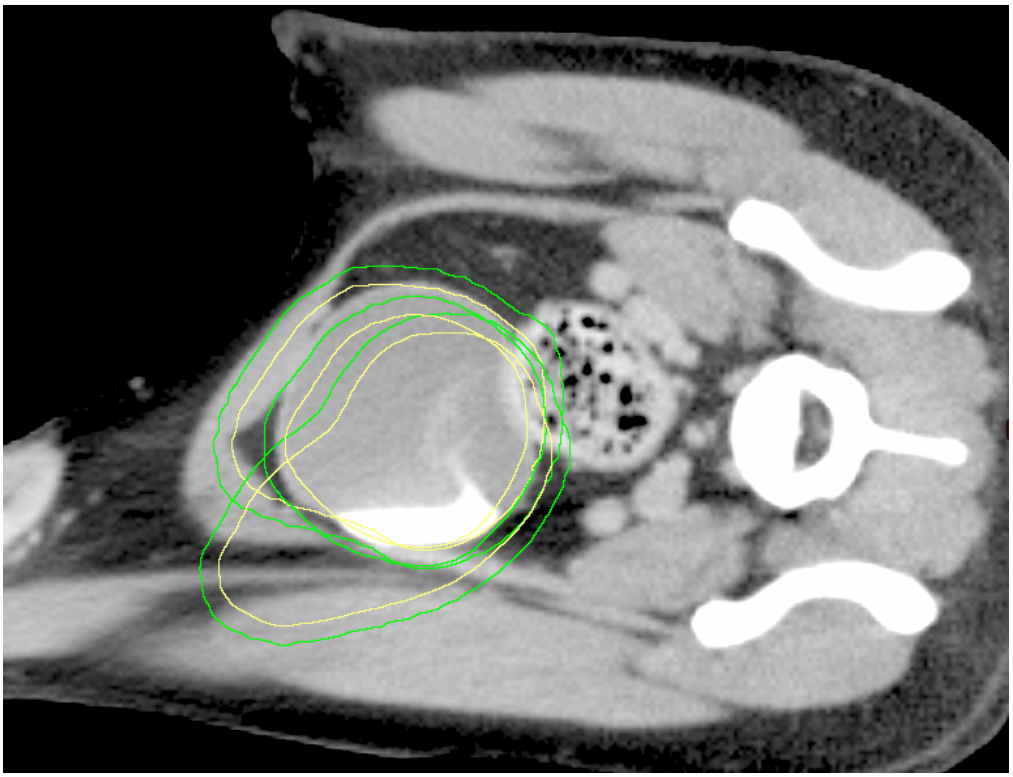


FIG. 3.3 Example of daily ART treatment volumes based on daily CBCT imaging. Daily bladder volumes (yellow contours) become the basis for each new daily PTV volume (green contours).

3.2f DVH Evaluation

Common dose constraints for the bladder and rectum were chosen to evaluate all plan types and enable the comparison of different plan types. The percentage of the bladder volume receiving at least 95% of the prescribed dose was obtained from the DVH to evaluate bladder coverage. This value was chosen based on International Commission on Radiation Units (ICRU) rules, common practices in the CSUVTH, and similar human studies[61, 63, 65]. The volume of rectum, in cc, receiving at least 95% and 100% of the prescribed dose was obtained to evaluate rectal irradiation. These percentages were chosen based on the similar human studies[61].

3.2g Statistics

Differences in dose delivered to the bladder and rectum were compared between parallel opposed, bony anatomy registration, soft tissue registration, and ART plan types. Results were analyzed by one-way ANOVA followed by Tukey's test for individual between-group comparisons. Statistical significance was assumed at $P < 0.05$.

All statistical analysis was performed using SAS statistical software v. 10.0 (SAS, Chicago, IL, USA).

3.3 Results

The potential improvement in dose distribution that could be gained by using more complex conformal planning techniques than a typical parallel

opposed plan (Section 2.3b) was evaluated. The use of all three advanced planning techniques (IMRT, IGRT and ART) showed a significantly lower volume of the rectum receiving 100% of the prescription dose when compared to parallel opposed plans ($p = 0.03$ for IMRT, $p < 0.001$ for IGRT, $p = 0.01$ for ART). Similarly, the use of all three advanced planning techniques (IMRT, IGRT and ART) showed a significantly lower volume of the rectum receiving 95% of the prescription dose when compared to parallel opposed plans ($p < 0.001$ for IMRT, $p = 0.005$ for IGRT, $p < 0.001$ for ART). ART allows for the greatest sparing of rectal tissue, with the lowest volume of rectum being irradiated using this technique ($p < 0.001$ for 100% and 95% of prescription dose). ART plans also have the largest volume of bladder receiving 95% of the prescription dose ($p < 0.001$). All dosimetric data was obtained from the TPS-generated DVH. Data is discussed in the following sections and is summarized in Table 3.1.

3.3a Bony Anatomy Registration

IMRT planning using kV CBCT-based bony anatomy registration showed 95% of the prescription dose to cover a mean bladder volume percentage ± 1 SD of 95.8% (range, 78.5 – 99.8%) ± 5.6% with 100% of the prescription dose being delivered to a mean rectal volume (cc) ± 1 SD of 3.3 ± 4.9 cc and 95% of the prescription dose delivered to a mean rectal volume (cc) ± 1 SD of 6.7 ± 7.7 cc.

3.3b Soft Tissue Registration

IMRT planning using kV CBCT-based soft tissue registration showed 95% of the prescription dose to cover a mean bladder volume percentage ± 1 SD of 97.3% (range, 92.0-100%) ± 2.5% with 100% of the prescription dose being delivered to a mean rectal volume (cc) ± 1 SD of 1.5 ± 2.2 cc and 95% of the prescription dose delivered to a mean rectal volume (cc) ± 1 SD of 4.4 ± 4.5 cc.

3.3c ART

ART IMRT planning using kV CBCT-based bony anatomy registration showed 95% of the prescription dose to cover a mean bladder volume percentage ± 1 SD of 100% (range, 100 – 100%) ± 0% with 100% of the prescription dose being delivered to a mean rectal volume (cc) ± 1 SD of 0.006 ± 0.01 cc and 95% of the prescription dose delivered to a mean rectal volume (cc) ± 1 SD of 0.4 ± 0.5 cc.

Table 3.1 Comparison of Dose to Bladder and Rectum using Advanced RT Techniques.

	Bony Anatomy[a]		*Soft Tissue*[a]		*ART*[a]	
	Mean (Range)	**Std Dev**	**Mean (Range)**	**Std Dev**	**Mean (Range)**	**Std Dev**
Bladder – 95%[b]	95.8 (78.5-99.8)	5.6	97.3 (92.0-100.0)	2.5	100.0 (100-100)	0.0
Rectum – 100%[c]	3.3 (0.01-20.7)	4.9	1.5 (0.0-8.3)	2.2	0.01 (0.0-0.07)	0.02
Rectum – 95%[d]	6.7 (0.6-34.6)	7.7	4.4 (0.07-19.7)	4.5	0.4 (0.0-1.5)	0.5

a. Advanced RT treatment technique used. 80 measurements per position (20 measurements for each of 4 dogs).

b. Percentage of bladder volume receiving 95% of prescription dose.

d. Rectal volume (cc) receiving 100% of prescription dose.

e. Rectal volume (cc) receiving 95% of prescription dose.

3.4 Discussion of Results

As the field of radiation oncology progresses, new techniques become available to improve the outcome of RT. Many of these have the ability to improve upon the standard techniques that are used for canine bladder cancer patients. Image guided techniques utilizing bony or soft tissue registration, as well as adaptive techniques were examined. Although none of these techniques are commonly used for the treatment of canine bladder cancer, results of this study show that all of these techniques could provide adequate bladder coverage while simultaneously reducing the dose received by the rectum (Figs. 3.4, 3.5). This dose reduction could translate to reduced treatment toxicity, in terms of both acute and late treatment effects and permit PTV dose escalation. It is thought that the combination of IMRT and IGRT has the potential to achieve both unparalleled tumor control and normal tissue sparing[5].

The treatment plans using IMRT and kV CBCT-based bony anatomy registration introduced a conformal technique that substantially reduced the amount of irradiated tissue from that which was irradiated with the parallel opposed plan. One hundred percent of the prescription dose was delivered to an average of only 3.3 ± 4.9 cc of the total rectal volume (vs.7.0 ± 7.9 cc of the total rectal volume received the total dose with the parallel opposed plan). However, with this dose conformality, care needs to be taken to direct the prescribed dose to the intended target. There is no simple way to account for organ motion each day and this motion can lead to inaccurate delivery of high radiation doses. If the conformal dose is not accurately delivered, geographic miss of the target or over-irradiation of the neighboring critical structures can occur.

An IMRT planning technique using kV CBCT-based soft tissue registration was next examined. Soft tissue registration provided an even more advantageous dose distribution with the rectal volume receiving 100% of the prescribed dose of 1.5 ± 2.2 cc. This could be particularly advantageous during RT for bladder cancer with respect to the close proximity of the bladder and rectum. The highly conformal doses that are used with IMRT need to be delivered to the appropriate location each day or the treatment outcome will be compromised.

The possibility of adapting the daily plan based on the CBCT images was examined. We accomplished this by using IMRT planning techniques that utilized kV CBCT-based bony anatomy registration. This type of ART planning showed the best dose distribution of all the plans we examined ($p < 0.001$), with 100% of each daily bladder volume receiving the full 54 Gy prescription dose and only 0.006 ± 0.01 cc of the total rectal volume receiving the entire prescribed dose. This type of adaptation requires a new plan to be made each day, so the practicality of using this type of RT in a clinical setting would need to be examined.

Possible downfalls to these types of image guided therapy would be the need for images of high-enough quality to easily visualize the entire bladder volume each day. This type of technology is not yet available to all facilities but it is growing. Another possible obstacle would be the need to train and familiarize radiation therapists with the soft tissue registration technique as the advanced imaging technology becomes available, as this is not a common practice in

veterinary facilities. Another consideration is the added dose from the daily CBCT imaging. The estimated dose of each CBCT is approximately 2-3 cGy[45], which would therefore add less than 1 Gy to the total treatment dose. However, this cannot be directly compared with a large treatment dose of 54 Gy because the treatment dose is conformed to the target volume only, while the CBCT dose includes the entire imaged volume. In addition, the imaging dose is in the kV range, while the treatment dose in the higher MV range. The risk of a second malignancy, however, is diminished by the typically older age of dogs with bladder cancer.

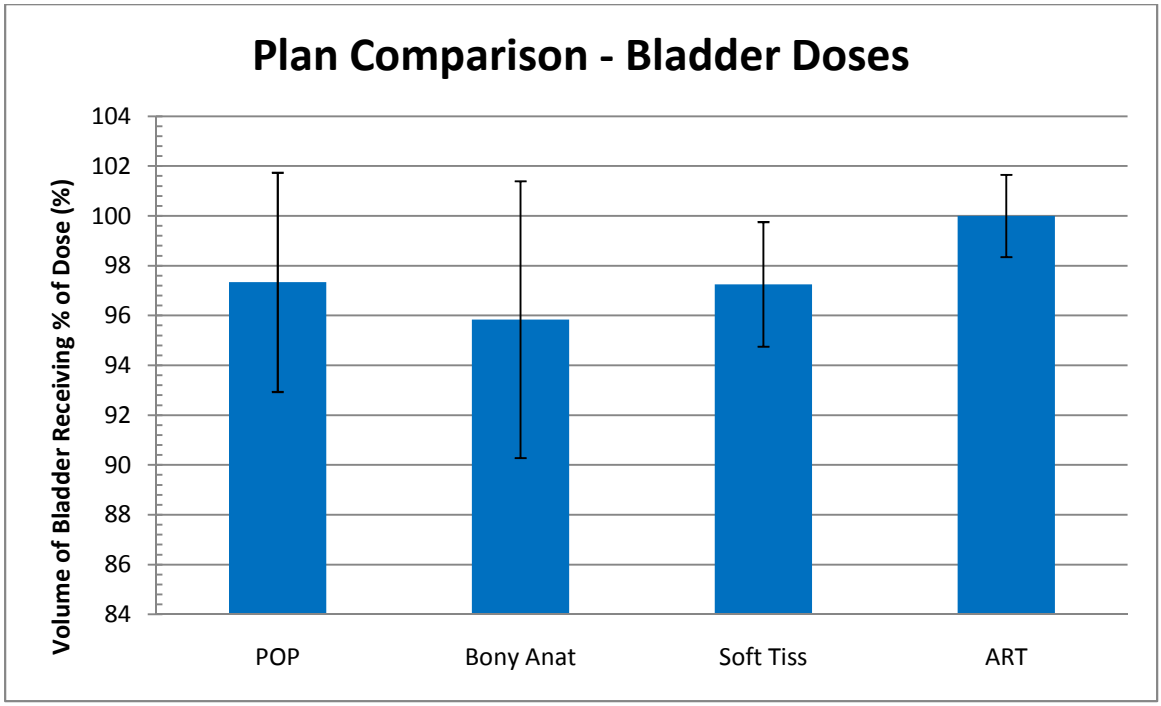


FIG. 3.4 Graph of average bladder volume (%) receiving at least 95% of 54 Gy prescribed dose for each plan type. Error bars are ± 2 standard deviations.

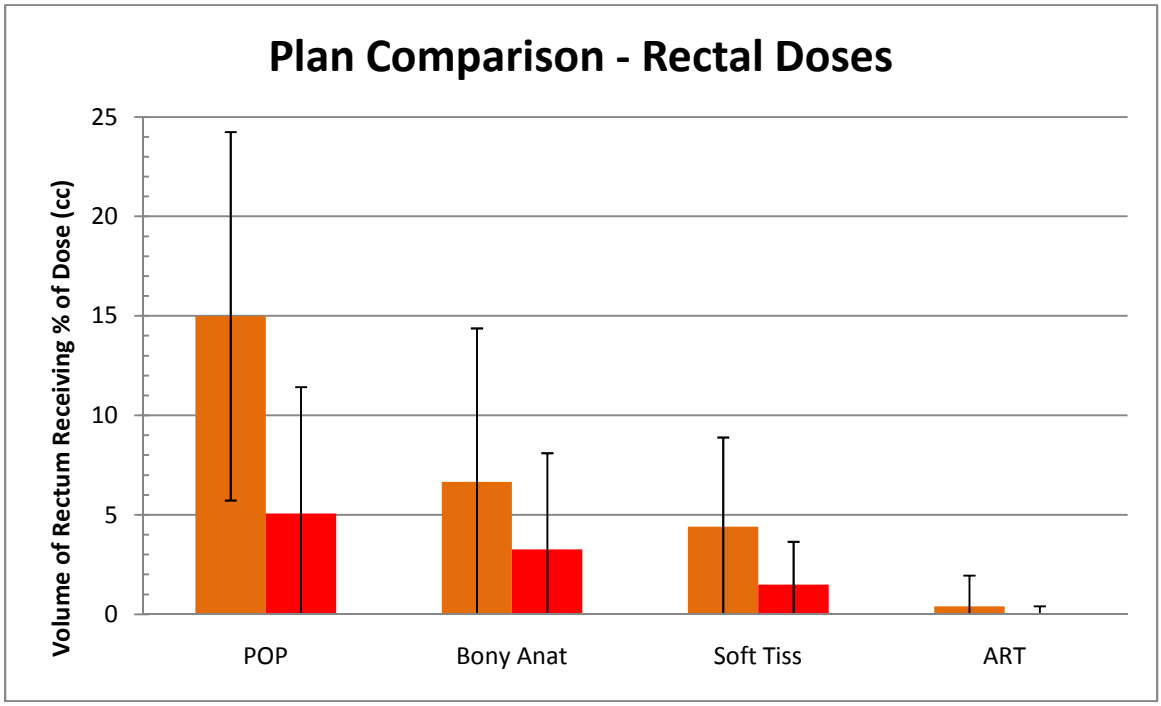


FIG. 3.5 Graph of average rectal volume (cc) receiving 95% (orange) and 100% (red) of 54 Gy prescribed dose for each plan type. Error bars are ± 2 standard deviations.

CHAPTER 4
GENERAL DISCUSSION and CONCLUSION

4.1 Summary and Discussion of Results

The nature of this study and the CBCT imaging technology allowed for the examination of the actual daily variations in position and treatment dose experienced by the canine bladder and allowed for the examination of different treatment scenarios using retrospective patient data. Characterization of the type of variation experienced by the canine bladder, determination of which treatment position provides the best dose distribution, and examination of the dose distributions resulting from three PTV expansions using a routine parallel-opposed treatment plan was performed. This information then allowed an evaluation of using more advanced RT treatment techniques for the treatment of canine bladder cancer. Sample plans were based on retrospective patient imaging data and provided insight into the possibility of using these types of techniques in a clinical setting.

There has been an increase in use of radiation oncology in veterinary medical practice over the past decade[1] with 69 clinics currently offering some form of external radiation therapy[4], ranging from linear accelerators to orthovoltage. However, treatment technologies and protocols differ from facility to facility[1]. The information from this study will allow clinics to make treatment

decisions based on actual patient data that will enable them to optimize treatment with the modalities available to them

4.2 Future Directions

4.2a Clinical Application of ART

The ability to assess positional differences on a daily basis and adapt the plan based on these variations enables more conformal treatment strategies to be implemented. Other than resource implications and imaging dose (Section 3.4), the only disadvantage to using such ART technology in a clinical setting is the time constraints. For each new ART plan created each day, the CBCT has to be transferred to the TPS and new contours have to be drawn by hand for the key organ(s) of interest, as well as a new PTV structure. A new IMRT plan has to be constructed that uses the new bladder volume PTV as an objective and this new plan has to be optimized. The dose would then have to be calculated and approved before it could be sent back to the treatment delivery machine and delivered to the patient.

An estimated minimum of 40 minutes would be needed to complete ART plans for each individual day of a treatment based on construction of our sample ART plans (Table 4.1). Additional planning time will add up in a busy clinic, increasing both clinical personnel time and canine patient time under anesthesia. The possibility for anesthesia-related complications will increase and may also increase the cost to owners. An ART plan that combines IMRT, IGRT and some

hybrid form of plan adaptation could be utilized to combat these time-related concerns.

Table 4.1 Estimated Time to Complete ART and Hybrid ART Planning

	ART	Hybrid ART
CBCT	5 min/day	5 min/day
Registration Type	Bony Anatomy	Soft Tissue
Registration	5 min/day	5 min/day
Contouring	10 min/day	N/A
Planning	10 min/day	2 min/day
Optimization	10 min/day	N/A
TOTAL TIME	**40 min/day**	**12 min/day**

4.2b Hybrid ART Plan

A hybrid planning technique was proposed that combined image guidance with a choice of pre-calculated plans to achieve the benefit of ART but without the time constraints. This type of planning technique may provide the tumor targeting and normal tissue sparing that is seen with ART. A hybrid approach eliminates the need to re-contour the bladder and rectal volumes each day and would therefore reduce the amount of time needed to develop an adaptive plan.

Hybrid ART utilizes pre-treatment CBCT to evaluate the patient position and bladder motion characteristics each day, immediately prior to treatment. The radiation therapist would then chose a plan from a library of plans that best fits that day's bladder volume. A soft tissue registration would be performed and the dose for the best-fitting plan would be delivered.

The bladder characterization data from Aim 1 of this study was used to develop the hybrid ART plans. Again, similar to the daily ART plans, only dogs in lateral recumbency were examined, as this was found to be the treatment position with the least interfractional variation. The feasibility of this technique was tested using a small (Fig. 4.1), medium (Fig. 4.2) and large plan (Fig. 4.3). Since bladder wall motion is most pronounced in the cranial and ventral directions, non-uniform margins were used to construct the target volumes for each of the plans (Table 4.2). The small plan applied a 5 mm PTV expansion in each of these directions to the original bladder volume, but did not add a margin in any of the other directions. This does not imply that there is no bladder wall motion in these directions, it simply means that the daily motion of each of the

other bladder walls fits within the original planning CT bladder volume. The medium plan applied a 15 mm expansion to the ventral wall, a 5 mm expansion to the cranial wall and a 3 mm expansion to the right, left, caudal and dorsal walls of the planning CT bladder volume. The large plan applied a 20 mm expansion to the ventral wall, a 10 mm expansion to the cranial wall, a 5 mm expansion to the right and left walls and a 3 mm expansion to the dorsal and caudal walls.

A 7-field IMRT plan using kV CBCT-based soft tissue registration was developed to deliver 54 Gy in 20 fractions of 2.7 Gy. We examined the DVH for each day using the same criteria as we did for the previous plans. This sample case of a hybrid ART plan provided a favorable dose distribution that fell between than that seen for IGRT with soft tissue registrations and ART. Our example hybrid ART plan using a soft tissue registration showed 95% of the prescription dose to cover a mean bladder volume percentage ± 1 SD of 97.6% (range, 94.0% – 99.9%) ± 1.7% with 100% of the prescription dose being delivered to a mean rectal volume (cc) ± 1 SD of 0.2 ± 0.4 cc and 95% of the prescription dose delivered to a mean rectal volume (cc) ± 1 SD of 1.2 ± 1.6 cc. The Hybrid RT plan is seen compared with the four other plan types in Figures 4.4 and 4.5.

Based on this data, it is feasible that a practical, hybrid ART technique utilizing CBCT-assisted plan selection could be developed to reduce the volume of rectum receiving high doses and continue to provide adequate coverage to the bladder volume. A hybrid ART technique would provide plan adaptation based on the patient anatomy each day, but it would reduce the time required to re-plan the treatment each day.

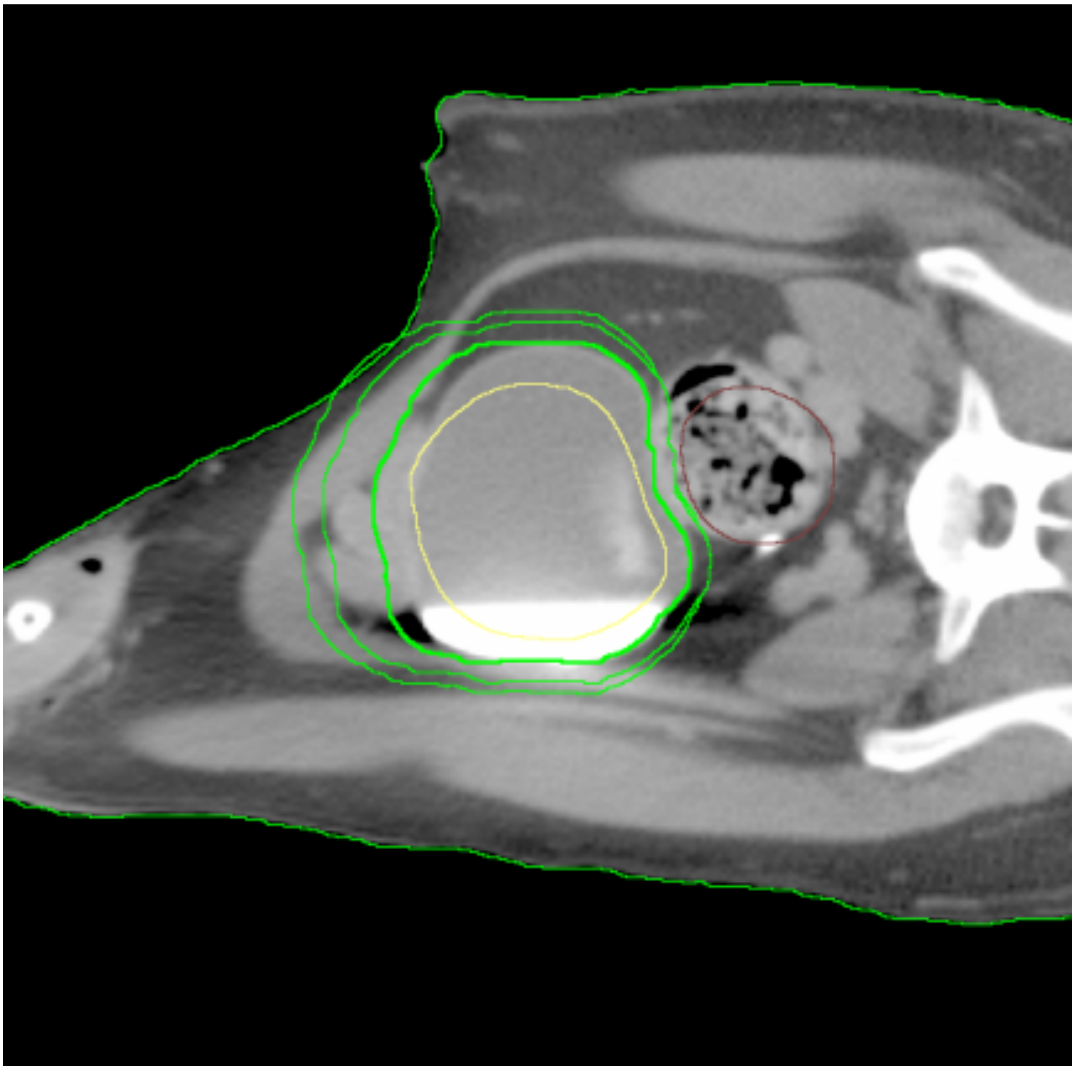


FIG. 4.1 Small, medium and large PTV volumes (green) shown on planning CT image with bladder volume (yellow) from one daily CBCT. The bladder volume fits within the small PTV (think green contour), so the small plan was chosen as the PTV for this day.

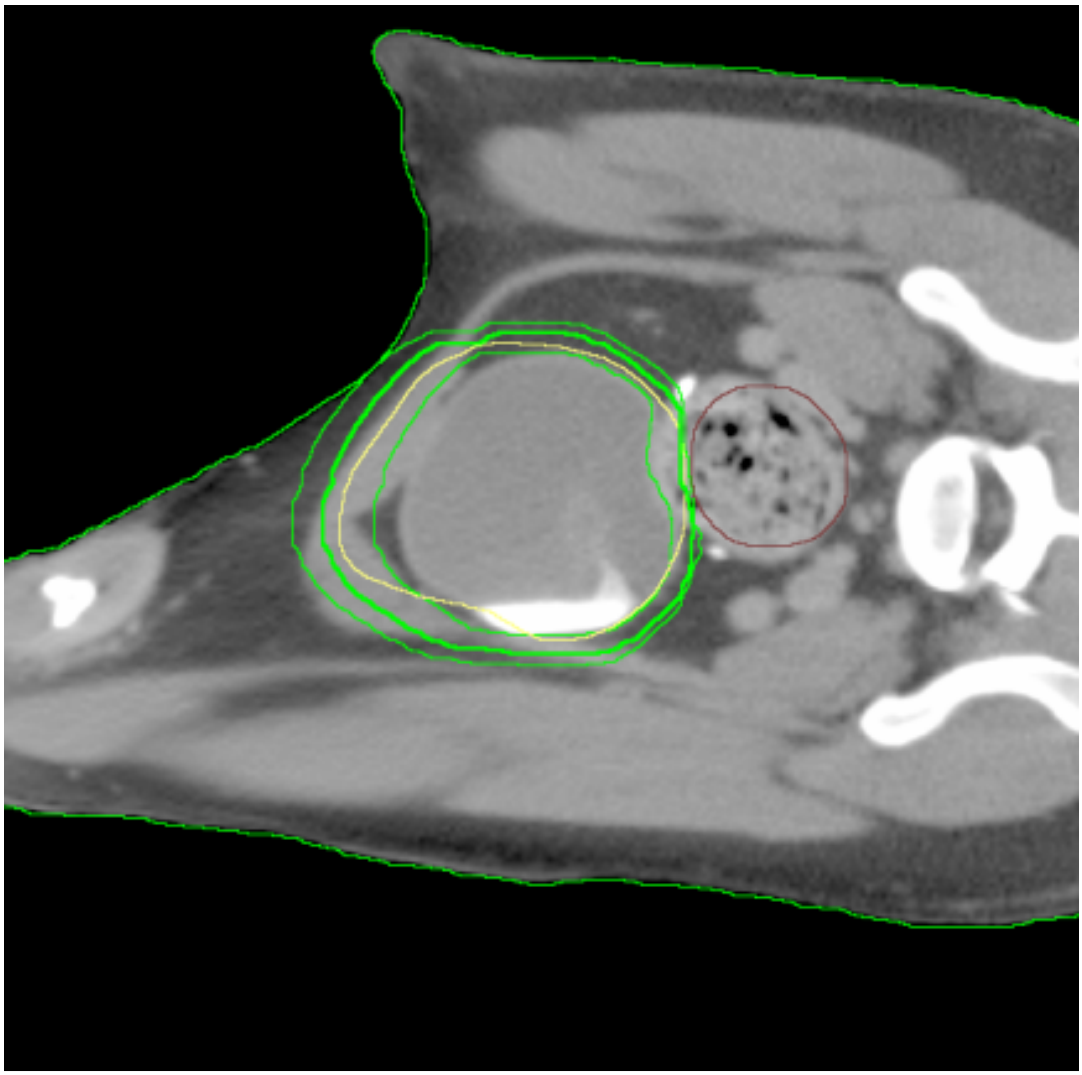


FIG. 4.2 Small, medium and large PTV volumes (green) shown on planning CT image with bladder volume (yellow) from one daily CBCT. The bladder volume fits within the medium PTV (think green contour), so the medium plan was chosen as the PTV for this day.

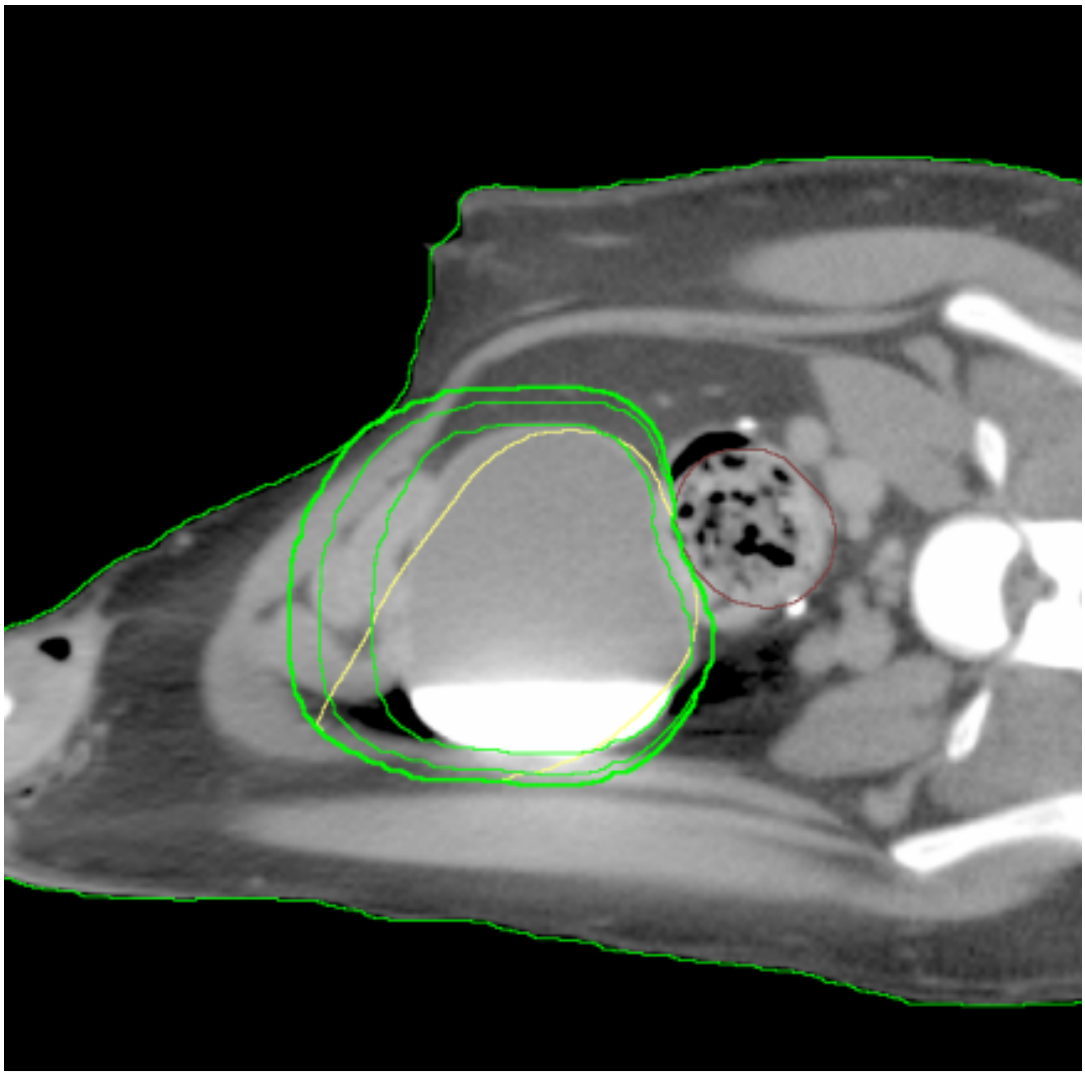


FIG. 4.3 Small, medium and large PTV volumes (green) shown on planning CT image with bladder volume (yellow) from one daily CBCT. The bladder volume fits within the large PTV (think green contour), so the large plan was chosen as the PTV for this day.

Table 4.2 Hybrid ART Margins (mm) Applied to Original Planning CT Bladder Volume in each Direction

	Small	Medium	Large
Right	0	3	5
Left	0	3	5
Dorsal	0	3	3
Ventral	5	15	2
Cranial	5	5	1
Caudal	0	3	3

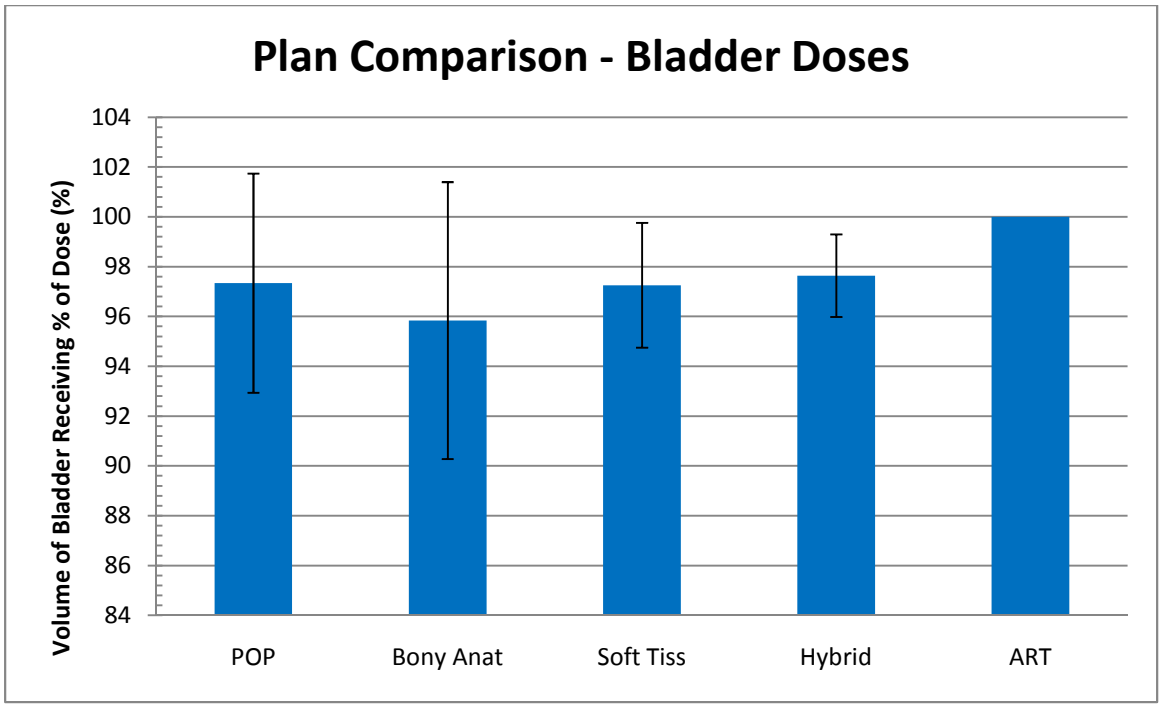


FIG 4.4 Graph of average bladder volume (%) receiving at least 95% of 54 Gy prescribed dose for each plan type. Error bars are ± 2 standard deviations.

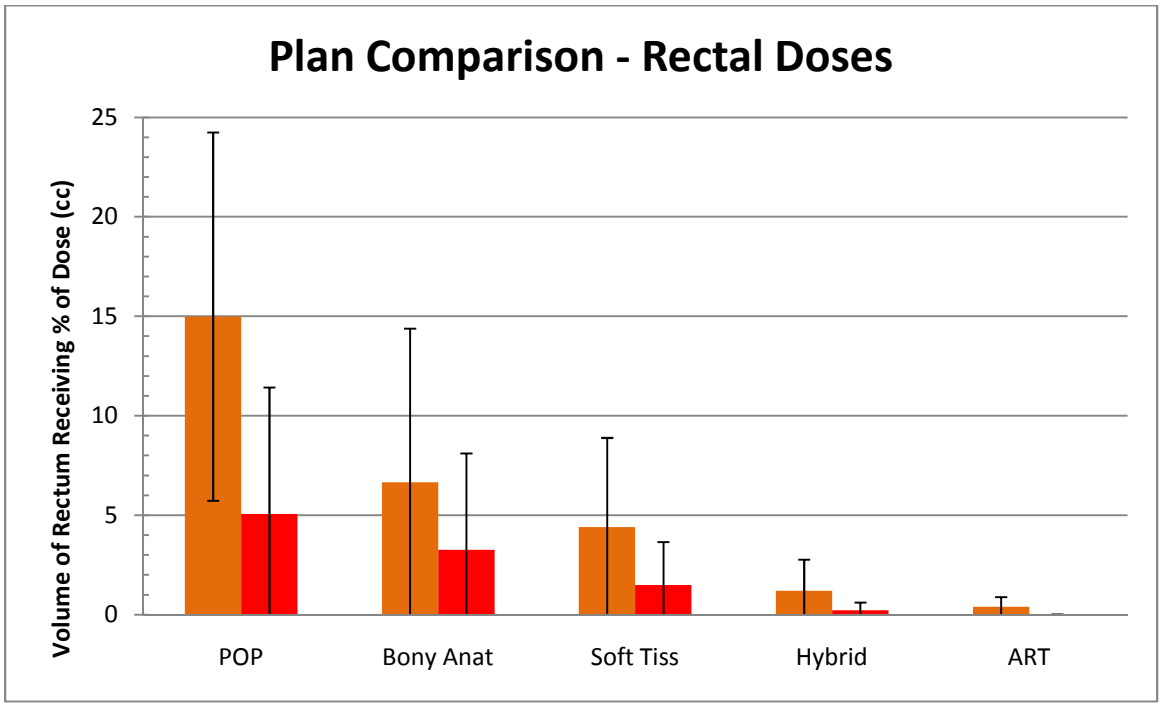


FIG. 4.5 Graph of average rectal volume (cc) receiving at least 95% (orange) and at least 100% (red) of 54 Gy prescribed dose for each plan type. Error bars are ± 2 standard deviations.

4.2c Proposed Clinical Trial

This study shows that volumetric information obtained from CBCT image data during the treatment of canine bladder cancer could be used to reduce the dose to critical organs, such as the colon and rectum. Many different planning techniques are feasible for the treatment of bladder cancer based on this retrospective data. Evaluation of these techniques in a clinical setting is needed to determine practicality and efficacy, and to create an optimal protocol for canine bladder cancer with more optimistic outcomes for patients.

4.3 Translational Medicine

Valid animal models are needed to test new cancer therapies. Animal models facilitate a better understanding of the molecular and biological processes involved in the tumor response, as well as provide information about treatment delivery and side effects. With more than 50,000 people diagnosed with bladder cancer each year in the United States[66], animal models could be helpful in developing a more effective treatment for this malignancy.

Canine TCC has been found to be similar to human TCC with regards to histopathology, biological behavior, metastasis, response to therapy, and prognosis[28] (Table 4.3). Pet dogs share the same environment as their owners, with exposure to the same water, air and chemicals. These similarities could make dogs a very effective model for human bladder TCC. Dogs are also of a larger size than most laboratory rodents and this makes testing of clinical procedures less difficult.

Table 4.3 Human and Canine TCC Similarities[28]

	Canine TCC	Human TCC
% of all cancers	1.5-2%	2%
mean age at diagnosis	11 yrs (60 human equiv. yrs)	65 yrs
environmental risk	increased risk in urban areas; increased risk with benzene exposure	increased risk in urban areas; increased risk with benzene exposure
histopathology	invasive; intermed. to high grade	invasive; intermed. to high grade
metastasis at diagnosis	20% of dogs	5-20% of patients
sites of metastasis	regional nodes and lung most common	regional nodes and lung most common

Not only do studies performed on dogs benefit humans, but advancements in human medicine can be used to improve the treatment of similar malignancies in veterinary patients. Information from human bladder cancer studies, combined with information from this study, could improve the treatment outcome for canine bladder TCC. Human studies have shown that higher total doses, up to 84 Gy, produce the best long term tumor control and survival rates[67]. Evidence that increasing the dose above current human standards (60–64 Gy with conventional fractionation) leads to improved local tumor control for bladder cancer has also been found in other clinical studies[67, 68].

Most late effects were documented in these human studies at total doses higher than 45-60 Gy. Dose escalation above current veterinary treatment standards may be a possible method to achieve better local tumor control for canine patients. However, to avoid overirradiation of the rectum and unwanted side effects, highly conformal doses need to be delivered using image guidance and appropriate PTV margins. The results and techniques examined in this study could be instrumental in optimizing bladder cancer treatment protocols.

4.4 Conclusions

Prior to this study, little information was available that characterized or quantified the motion experienced by the canine bladder on a daily basis, throughout the course of fractionated RT. The results of this study have allowed us to quantify this variation using real patient images and anatomical data, and to

recommend the treatment set-up that will minimize these variations. This study has also allowed us to examine the effects these interfractional bladder variations would have on model dose distributions for different treatment planning techniques.

4.4a Characterization of Bladder Variations

Aim 1 of this study used CBCT imaging technology to:

1. quantify the daily motion characteristics of the canine urinary bladder in three different treatment positions, and
2. recommend a PTV expansion margin that would ensure adequate irradiation of the entire bladder each day, while minimizing irradiation of the rectum, leading to unacceptable complications.

Quantification of the daily bladder wall position variations experienced by the canine urinary bladder on a daily basis with dogs in three possible treatment positions was done by making distance measurements in six directions. These data were then used to determine that dogs in lateral recumbency showed the least amount of bladder wall variation, and therefore the most advantageous treatment position.

In addition to taking bladder variation measurements, a 5, 10 or 15 mm treatment margin was added to the planning CT bladder volume. Sample treatment plans were developed and dosimetric data for each plan was then

examined. The recommended 10 mm PTV expansion ensured adequate irradiation of the entire bladder each day while minimizing the dose received by the rectum.

4.4b Optimization of Bladder Cancer Radiation Therapy using Motion Data

Aim 2 of this study used the bladder motion data from Aim 1 to:

1. develop plans and compare dosimetric data for different advanced RT techniques, and
2. develop an adaptive RT (ART) protocol that would optimize the dose delivery for canine bladder cancer.

The bladder motion and treatment setup data from Aim 1 was used to create different types of advanced RT plans. Advanced plan types evaluated included an intensity modulated RT (IMRT) plan using bony anatomy registration and an IMRT plan using soft tissue registration. The feasibility of an ART plan that used a new target volume based on each day's anatomy was also examined. The dosimetric data for the new plans, as well as the dosimetric data from the parallel opposed plans in Aim 1, was compared using DVH's and all advanced planning techniques showed a lower dose to the rectal volume. The optimal treatment protocol was found to be the ART plan that provided the most

conformal, curative-intent dose to the bladder while simultaneously providing the lowest dose to the rectum.

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APPENDIX

LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ART	Adaptive Radiotherapy
CBCT	Cone Beam Computed Tomography
cc	Cubic Centimeters
CRT	Conformal Radiation Therapy
CSU	Colorado State University
CSUVTH	Colorado State University Veterinary Teaching Hospital
CT	Computed Tomography
CTV	Clinical Target Volume
DART	Dynamic Adaptive Radiotherapy
DVH	Dose Volume Histogram
GTV	Gross Target Volume
Gy	Gray
IGRT	Image Guided Radiotherapy
IMRT	Intenisty Modulated Radiotherapy
kV	Kiolovoltage
mA	Miliamps
MeV	Mega Electronvolts
mm	Millimeter
MV	Megavoltage
NSAID	Non-steroidal Anti-inflammatory Drugs
PTV	Planning Target Volume
RT	Radiotherapy/Radiation Therapy
SD	Standard Deviation
TCC	Transitional Cell Carcinoma
TPS	Treatment Planning System

SAS STATISTICAL ANALYSIS OUTPUT

Dependent variable = Rt 13:54
Friday, July 23, 2010 165

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	Rt
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-34.83949419	
1	2	-36.93967041	0.00138316

	2	1	-37.13693930	0.00024404
	3	1	-37.16892209	0.00000922
4		1	-37.17003714	0.00000001
	5	1	-37.17003889	0.00000000

Convergence criteria met.

Dependent variable = Rt 13:54
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The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	0.007822
Residual	0.03495

Fit Statistics

-2 Res Log Likelihood	-37.2
AIC (smaller is better)	-33.2
AICC (smaller is better)	-33.1
BIC (smaller is better)	-32.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	3.16	**0.0465**

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	1.0024	0.05828	101	17.20	<.0001
Position	2	0.9026	0.05790	101	15.59	<.0001
Position	3	0.7858	0.06341	101	12.39	<.0001

Differences of Least Squares Means

Standard

Effect	Position	Position	Estimate	Error	DF	t Value	Pr > |t|
Position	1	2	0.09976	0.08215	101	1.21	0.2274
Position	**1**	**3**	**0.2166**	**0.08612**	**101**	**2.51**	**0.0135**
Position	2	3	0.1168	0.08587	101	1.36	0.1767

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	Lt
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	0.02882730	
1	2	-15.61009005	0.00000093
2	1	-15.61019037	0.00000000

Convergence criteria met.

Dependent variable = Lt 13:54
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The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	0.01771
Residual	0.04143

Fit Statistics

-2 Res Log Likelihood	-15.6
AIC (smaller is better)	-11.6
AICC (smaller is better)	-11.5
BIC (smaller is better)	-11.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	0.41	0.6644

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	0.8703	0.08271	101	10.52	<.0001
Position	2	0.9511	0.07912	101	12.02	<.0001
Position	3	0.9718	0.08746	101	11.11	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-0.08085	0.1145	101	-0.71	0.4816

Position 1 3 -0.1015 0.1204 101 -0.84 0.4010
Position 2 3 -0.02067 0.1179 101 -0.18 0.8612
Dependent variable = Dor 13:54
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The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	Dor
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	15.44848144	
1	3	-5.96124296	0.00035421
2	1	-6.00134708	0.00001223
3	1	-6.00262787	0.00000002
4	1	-6.00262964	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	0.02143
Residual	0.04505

Fit Statistics

-2 Res Log Likelihood	-6.0
AIC (smaller is better)	-2.0
AICC (smaller is better)	-1.9
BIC (smaller is better)	-1.4

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	0.96	0.3875

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	1.0497	0.09034	101	11.62	<.0001
Position	2	1.0874	0.08590	101	12.66	<.0001
Position	3	0.9160	0.09514	101	9.63	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-0.03763	0.1247	101	-0.30	0.7634
Position	1	3	0.1337	0.1312	101	1.02	0.3104
Position	2	3	0.1714	0.1282	101	1.34	0.1842

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	Ven
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	47.31118084	
1	5	7.00383917	0.00011255
2	1	6.99400249	0.00000158
3	1	6.99385032	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	0.04318
Residual	0.04917

Fit Statistics

-2 Res Log Likelihood	7.0
AIC (smaller is better)	11.0
AICC (smaller is better)	11.1
BIC (smaller is better)	11.6

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	1.86	0.1617

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	0.7867	0.1245	101	6.32	<.0001
Position	2	0.6788	0.1148	101	5.91	<.0001
Position	3	1.0080	0.1286	101	7.84	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	0.1080	0.1693	101	0.64	0.5252
Position	1	3	-0.2212	0.1790	101	-1.24	0.2194
Position	2	3	-0.3292	0.1724	101	-1.91	0.0590

Model Information

Data Set	WORK.TEMP
Dependent Variable	Cra
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	179.52788948	
1	3	126.55791552	0.00103777
2	1	126.51656860	0.00003597
3	1	126.51524259	0.00000005
4	1	126.51524073	0.00000000

Convergence criteria met.

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate

Dog	0.1873
Residual	0.1455

Fit Statistics

-2 Res Log Likelihood	126.5
AIC (smaller is better)	130.5
AICC (smaller is better)	130.6
BIC (smaller is better)	131.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	1.47	0.2345

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	0.6386	0.2564	101	2.49	0.0144
Position	2	1.2229	0.2326	101	5.26	<.0001
Position	3	0.8629	0.2624	101	3.29	0.0014

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-0.5843	0.3462	101	-1.69	0.0945
Position	1	3	-0.2243	0.3669	101	-0.61	0.5423
Position	2	3	0.3599	0.3507	101	1.03	0.3071

Dependent variable = Cau 13:54
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The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	Cau

Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	-111.86993398	
1	3	-120.51007284	0.00086303
2	1	-120.67170972	0.00009492
3	1	-120.68802161	0.00000154
4	1	-120.68826953	0.00000000

Convergence criteria met.

Dependent variable = Cau 13:54
Friday, July 23, 2010 176

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	0.003510
Residual	0.01615

Fit Statistics

-2 Res Log Likelihood	-120.7
AIC (smaller is better)	-116.7
AICC (smaller is better)	-116.6
BIC (smaller is better)	-116.1

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	5.54	**0.0052**

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	0.9914	0.03917	101	25.31	<.0001
Position	2	0.9253	0.03898	101	23.74	<.0001
Position	3	0.8002	0.04268	101	18.75	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	0.06611	0.05526	101	1.20	0.2344
Position	**1**	**3**	**0.1912**	**0.05793**	**101**	**3.30**	**0.0013**
Position	**2**	**3**	**0.1251**	**0.05780**	**101**	**2.16**	**0.0328**

Dependent variable = __5B95 13:54
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The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__5B95
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	713.57984224	
1	2	697.42979502	0.00000199
2	1	697.42929167	0.00000000

Convergence criteria met.

Dependent variable = __5B95 13:54
Friday, July 23, 2010 178

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	13.7638
Residual	30.4400

Fit Statistics

-2 Res Log Likelihood	697.4
AIC (smaller is better)	701.4
AICC (smaller is better)	701.5
BIC (smaller is better)	702.0

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	2.50	0.0869

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	91.1067	2.2969	101	39.66	<.0001
Position	2	98.1543	2.1903	101	44.81	<.0001
Position	3	95.5891	2.4237	101	39.44	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	**1**	**2**	**-7.0476**	**3.1739**	**101**	**-2.22**	**0.0286**
Position	1	3	-4.4824	3.3392	101	-1.34	0.1825
Position	2	3	2.5652	3.2668	101	0.79	0.4342

Dependent variable = __5R100 13:54 Friday, July 23, 2010 179

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__5R100
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3

Dog 10 1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	799.52569595	
1	3	770.15960185	0.00032319
2	1	770.05332561	0.00002472
3	1	770.04587885	0.00000019
4	1	770.04582529	0.00000000

Convergence criteria met.

Dependent variable = __5R100 13:54
Friday, July 23, 2010 180

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	37.7227
Residual	58.5455

Fit Statistics

-2 Res Log Likelihood	770.0
AIC (smaller is better)	774.0
AICC (smaller is better)	774.2
BIC (smaller is better)	774.7

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F

Position	2	101	0.84	0.4338

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	10.9865	3.7281	101	2.95	0.0040
Position	2	12.2353	3.4876	101	3.51	0.0007
Position	3	5.7316	3.8850	101	1.48	0.1432

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-1.2488	5.1051	101	-0.24	0.8072
Position	1	3	5.2549	5.3844	101	0.98	0.3314
Position	2	3	6.5037	5.2208	101	1.25	0.2157

Dependent variable = __0B95 13:54 Friday, July 23, 2010 181

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__0B95
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2

Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	632.56388697	
1	3	617.24617895	0.00011082
2	1	617.22086920	0.00000260
3	1	617.22031595	0.00000000

Convergence criteria met.

Dependent variable = __0B95 13:54
Friday, July 23, 2010 182

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	5.3078
Residual	14.6416

Fit Statistics

-2 Res Log Likelihood	617.2
AIC (smaller is better)	621.2
AICC (smaller is better)	621.3
BIC (smaller is better)	621.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	1.67	0.1929

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	95.3936	1.4492	101	65.83	<.0001
Position	2	98.9909	1.3996	101	70.73	<.0001
Position	3	97.9845	1.5428	101	63.51	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-3.5972	2.0147	101	-1.79	0.0772
Position	1	3	-2.5908	2.1167	101	-1.22	0.2238
Position	2	3	1.0064	2.0830	101	0.48	0.6300

Dependent variable = __0R100 13:54
Friday, July 23, 2010 183

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__0R100
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1

Max Obs Per Subject 111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	829.65339723	
1	3	810.21903034	0.00004641
2	1	810.20379669	0.00000067
3	1	810.20358820	0.00000000

Convergence criteria met.

Dependent variable = __0R100 13:54
Friday, July 23, 2010 184

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	38.4526
Residual	86.5609

Fit Statistics

-2 Res Log Likelihood	810.2
AIC (smaller is better)	814.2
AICC (smaller is better)	814.3
BIC (smaller is better)	814.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	0.43	0.6513

Least Squares Means

Standard

Effect	Position	Estimate	Error	DF	t Value	Pr > |t|
Position	1	14.2525	3.8437	101	3.71	0.0003
Position	2	17.5986	3.6690	101	4.80	<.0001
Position	3	12.6974	4.0587	101	3.13	0.0023

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-3.3461	5.3137	101	-0.63	0.5303
Position	1	3	1.5550	5.5899	101	0.28	0.7814
Position	2	3	4.9012	5.4712	101	0.90	0.3725

Dependent variable = __5B950 13:54
Friday, July 23, 2010 185

The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__5B950
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read 111
Number of Observations Used 111
Number of Observations Not Used 0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	541.57063759	
1	3	526.39526192	0.00090419
2	1	526.22079006	0.00010363
3	1	526.20244756	0.00000184
4	1	526.20214234	0.00000000

Convergence criteria met.

Dependent variable = __5B950 13:54
Friday, July 23, 2010 186

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	2.1145
Residual	6.3278

Fit Statistics

-2 Res Log Likelihood	526.2
AIC (smaller is better)	530.2
AICC (smaller is better)	530.3
BIC (smaller is better)	530.8

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	1.30	0.2775

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	97.5807	0.9207	101	105.98	<.0001

Position	2	99.4852	0.8935	101	111.35	<.0001
Position	3	99.2977	0.9837	101	100.95	<.0001

Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-1.9045	1.2830	101	-1.48	0.1408
Position	1	3	-1.7170	1.3473	101	-1.27	0.2055
Position	2	3	0.1875	1.3289	101	0.14	0.8881

Dependent variable = __5R1000 13:54
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The Mixed Procedure

Model Information

Data Set	WORK.TEMP
Dependent Variable	__5R1000
Covariance Structure	Variance Components
Estimation Method	REML
Residual Variance Method	Profile
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Position	3	1 2 3
Dog	10	1 2 3 4 5 6 7 8 9 10

Dimensions

Covariance Parameters	2
Columns in X	4
Columns in Z	10
Subjects	1
Max Obs Per Subject	111

Number of Observations

Number of Observations Read	111
Number of Observations Used	111
Number of Observations Not Used	0

Iteration History

Iteration	Evaluations	-2 Res Log Like	Criterion
0	1	874.42208504	
1	2	853.07143640	0.00000004
2	1	853.07142244	0.00000000

Convergence criteria met.

Dependent variable = __5R1000 13:54
Friday, July 23, 2010 188

The Mixed Procedure

Covariance Parameter Estimates

Cov Parm	Estimate
Dog	70.0212
Residual	127.35

Fit Statistics

-2 Res Log Likelihood	853.1
AIC (smaller is better)	857.1
AICC (smaller is better)	857.2
BIC (smaller is better)	857.7

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Position	2	101	1.83	0.1655

Least Squares Means

Effect	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	17.8587	5.1205	101	3.49	0.0007
Position	2	30.8488	4.8304	101	6.39	<.0001
Position	3	21.4827	5.3646	101	4.00	0.0001

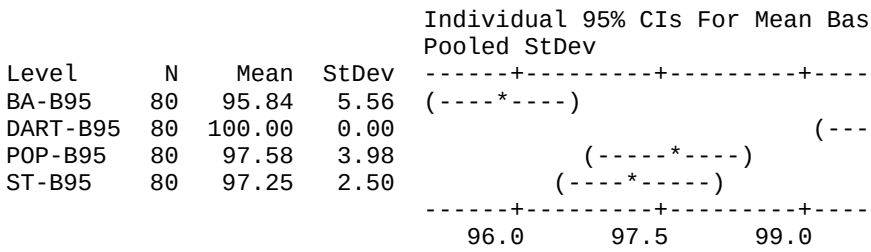
Differences of Least Squares Means

Effect	Position	Position	Estimate	Standard Error	DF	t Value	Pr > |t|
Position	1	2	-12.9901	7.0394	101	-1.85	0.0679
Position	1	3	-3.6240	7.4161	101	-0.49	0.6261
Position	2	3	9.3661	7.2188	101	1.30	0.1974

One-way ANOVA: C5 versus C6

Source	DF	SS	MS	F	P
C6	3	716.7	238.9	18.04	0.000
Error	316	4184.2	13.2		
Total	319	4900.9			

S = 3.639 R-Sq = 14.62% R-Sq(adj) = 13.81%



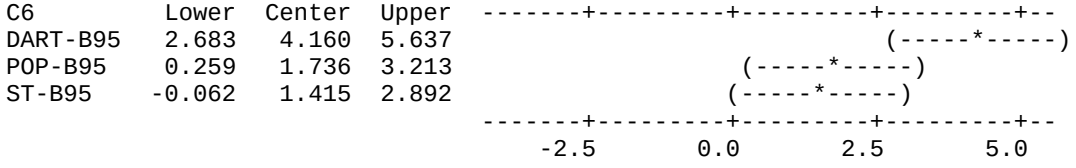
Pooled StDev = 3.64

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C6

Individual confidence level = 98.93%

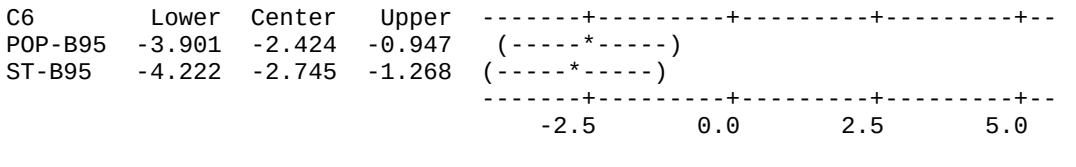
C6 = BA-B95 subtracted from:

C6	Lower	Center	Upper
DART-B95	2.683	4.160	5.637
POP-B95	0.259	1.736	3.213
ST-B95	-0.062	1.415	2.892



C6 = DART-B95 subtracted from:

C6	Lower	Center	Upper
POP-B95	-3.901	-2.424	-0.947
ST-B95	-4.222	-2.745	-1.268



```
C6 = POP-B95 subtracted from:

C6       Lower  Center  Upper  -------+---------+---------+---------+--
ST-B95  -1.798  -0.321  1.156           (-----*-----)
                               -------+---------+---------+---------+--
                                   -2.5       0.0       2.5       5.0

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-B95  subtracted from:

C6           Lower  Center  Upper  ----+---------+---------+---------+--
DART-B95   2.68320   4.160  5.637                        (----*----)
POP-B95    0.25945   1.736  3.213                 (----*----)
ST-B95    -0.06180   1.415  2.892                (----*----)
                               ----+---------+---------+---------+--
                                -3.0       0.0       3.0       6.0


C6 = DART-B95  subtracted from:

C6        Lower  Center   Upper  ----+---------+---------+---------+--
POP-B95  -3.901  -2.424  -0.947   (----*----)
ST-B95   -4.222  -2.745  -1.268  (----*----)
                               ----+---------+---------+---------+--
                                -3.0       0.0       3.0       6.0


C6 = POP-B95  subtracted from:

C6       Lower   Center  Upper  ----+---------+---------+---------+--
ST-B95  -1.798  -0.3213  1.156          (----*----)
                               ----+---------+---------+---------+--
                                -3.0       0.0       3.0       6.0


Tukey Simultaneous Tests
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-B95  subtracted from:

          Difference       SE of           Adjusted
C6          of Means  Difference  T-Value   P-Value
DART-B95       4.160      0.5753    7.230    0.0000
POP-B95        1.736      0.5753    3.018    0.0136
ST-B95         1.415      0.5753    2.459    0.0664


C6 = DART-B95  subtracted from:

         Difference       SE of           Adjusted
C6         of Means  Difference  T-Value   P-Value
POP-B95      -2.424      0.5753   -4.213    0.0001
ST-B95       -2.745      0.5753   -4.771    0.0000


C6 = POP-B95  subtracted from:

        Difference       SE of           Adjusted
C6        of Means  Difference  T-Value   P-Value
ST-B95     -0.3213      0.5753  -0.5584    0.9443
```

One-way ANOVA: C5 versus C6

```
Source   DF       SS      MS      F      P
C6        3   9102.9  3034.3  73.11  0.000
Error   316  13114.1    41.5
Total   319  22217.0

S = 6.442   R-Sq = 40.97%   R-Sq(adj) = 40.41%


                            Individual 95% CIs For Mean Based on
                            Pooled StDev
Level      N    Mean  StDev  --+---------+---------+---------+-------
BA-R95    80   6.650  7.733             (--*--)
DART-R95  80   0.390  0.508  (--*--)
POP-R95   80  14.990  9.260                               (--*--)
ST-R95    80   4.403  4.494          (--*--)
                            --+---------+---------+---------+-------
                            0.0       5.0      10.0      15.0

Pooled StDev = 6.442


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C6

Individual confidence level = 98.93%


C6 = BA-R95 subtracted from:

C6         Lower  Center   Upper  -------+---------+---------+---------+--
DART-R95  -8.875  -6.260  -3.646          (--*-)
POP-R95    5.726   8.340  10.954                        (-*--)
ST-R95    -4.861  -2.246   0.368              (--*-)
                                -------+---------+---------+---------+--
                                     -10         0        10        20


C6 = DART-R95 subtracted from:

C6        Lower  Center   Upper  -------+---------+---------+---------+--
POP-R95  11.986  14.600  17.215                                (--*-)
ST-R95    1.399   4.014   6.628                    (--*--)
                                -------+---------+---------+---------+--
                                     -10         0        10        20


C6 = POP-R95 subtracted from:

C6        Lower   Center   Upper  -------+---------+---------+---------+--
ST-R95  -13.201  -10.587  -7.972      (-*--)
                                -------+---------+---------+---------+--
                                     -10         0        10        20
```

General Linear Model: C5 versus C6

```
Factor  Type   Levels  Values
C6      fixed       4  BA-R95, DART-R95, POP-R95, ST-R95
```

Analysis of Variance for C5, using Adjusted SS for Tests

Source	DF	Seq SS	Adj SS	Adj MS	F	P
C6	3	9102.9	9102.9	3034.3	73.11	0.000
Error	316	13114.1	13114.1	41.5		
Total	319	22217.0				

S = 6.44208 R-Sq = 40.97% R-Sq(adj) = 40.41%

Unusual Observations for C5

Obs	C5	Fit	SE Fit	Residual	St Resid	
4	32.4000	14.9900	0.7202	17.4100	2.72	R
20	45.1000	14.9900	0.7202	30.1100	4.70	R
29	32.4000	14.9900	0.7202	17.4100	2.72	R
39	45.1000	14.9900	0.7202	30.1100	4.70	R
44	32.4000	14.9900	0.7202	17.4100	2.72	R
48	45.1000	14.9900	0.7202	30.1100	4.70	R
63	45.1000	14.9900	0.7202	30.1100	4.70	R
67	32.4000	14.9900	0.7202	17.4100	2.72	R
84	19.5000	6.6500	0.7202	12.8500	2.01	R
100	34.6000	6.6500	0.7202	27.9500	4.37	R
104	19.5000	6.6500	0.7202	12.8500	2.01	R
120	34.6000	6.6500	0.7202	27.9500	4.37	R
124	19.5000	6.6500	0.7202	12.8500	2.01	R
128	34.6000	6.6500	0.7202	27.9500	4.37	R
143	34.6000	6.6500	0.7202	27.9500	4.37	R
147	19.5000	6.6500	0.7202	12.8500	2.01	R
180	19.7000	4.4035	0.7202	15.2965	2.39	R
200	19.7000	4.4035	0.7202	15.2965	2.39	R
208	19.7000	4.4035	0.7202	15.2965	2.39	R
223	19.7000	4.4035	0.7202	15.2965	2.39	R

R denotes an observation with a large standardized residual.

Least Squares Means for C5

C6	Mean	SE Mean
BA-R95	6.6500	0.7202
DART-R95	0.3898	0.7202
POP-R95	14.9900	0.7202
ST-R95	4.4035	0.7202

Tukey 95.0% Simultaneous Confidence Intervals
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-R95 subtracted from:

C6	Lower	Center	Upper	---+---------+---------+---------+---
DART-R95	-8.875	-6.260	-3.646	(--*-)
POP-R95	5.726	8.340	10.954	(-*--)
ST-R95	-4.861	-2.246	0.368	(--*-)
				---+---------+---------+---------+---
				-10 0 10 20

C6 = DART-R95 subtracted from:

C6	Lower	Center	Upper	---+---------+---------+---------+---

```
POP-R95  11.986  14.600  17.215                            (--*-)
ST-R95    1.399   4.014   6.628                 (--*--)
                                  ---+---------+---------+---------+---
                                   -10         0        10        20


C6 = POP-R95  subtracted from:

C6       Lower  Center   Upper  ---+---------+---------+---------+---
ST-R95  -13.20  -10.59  -7.972  (-*--)
                                  ---+---------+---------+---------+---
                                   -10         0        10        20


Tukey Simultaneous Tests
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-R95  subtracted from:

          Difference       SE of           Adjusted
C6          of Means  Difference  T-Value   P-Value
DART-R95      -6.260       1.019   -6.146    0.0000
POP-R95        8.340       1.019    8.188    0.0000
ST-R95        -2.246       1.019   -2.206    0.1217


C6 = DART-R95  subtracted from:

         Difference       SE of           Adjusted
C6         of Means  Difference  T-Value   P-Value
POP-R95      14.600       1.019   14.334    0.0000
ST-R95        4.014       1.019    3.941    0.0005


C6 = POP-R95  subtracted from:

        Difference       SE of           Adjusted
C6        of Means  Difference  T-Value   P-Value
ST-R95      -10.59       1.019   -10.39    0.0050
```

One-way ANOVA: C5 versus C6

```
Source   DF      SS     MS      F      P
C6        3  1150.5  383.5  22.29  0.000
Error   316  5437.6   17.2
Total   319  6588.1

S = 4.148   R-Sq = 17.46%   R-Sq(adj) = 16.68%


                           Individual 95% CIs For Mean Based on
                           Pooled StDev
Level       N   Mean  StDev  -----+---------+---------+---------+----
BA-R100    80  3.256  4.855                  (---*----)
DART-R100  80  0.006  0.016  (----*----)
POP-R100   80  5.065  6.370                           (---*----)
ST-R100    80  1.490  2.165         (---*----)
                              -----+---------+---------+---------+----
                                 0.0       2.0       4.0       6.0

Pooled StDev = 4.148
```

```
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of C6

Individual confidence level = 98.93%


C6 = BA-R100 subtracted from:

C6          Lower  Center   Upper  ---------+---------+---------+---------+
DART-R100  -4.933  -3.250  -1.566       (----*----)
POP-R100    0.125   1.809   3.493                    (----*----)
ST-R100    -3.450  -1.766  -0.083           (----*----)
                                   ---------+---------+---------+---------+
                                         -3.5       0.0       3.5       7.0


C6 = DART-R100 subtracted from:

C6          Lower  Center  Upper  ---------+---------+---------+---------+
POP-R100    3.375   5.059  6.742                              (---*----)
ST-R100    -0.200   1.483  3.167                    (----*----)
                                  ---------+---------+---------+---------+
                                        -3.5       0.0       3.5       7.0


C6 = POP-R100 subtracted from:

C6         Lower  Center   Upper  ---------+---------+---------+---------+
ST-R100   -5.259  -3.575  -1.892      (----*----)
                                  ---------+---------+---------+---------+
                                        -3.5       0.0       3.5       7.0
```

General Linear Model: C5 versus C6

```
Factor  Type   Levels  Values
C6      fixed       4  BA-R100, DART-R100, POP-R100, ST-R100


Analysis of Variance for C5, using Adjusted SS for Tests

Source   DF   Seq SS   Adj SS  Adj MS      F      P
C6        3  1150.53  1150.53  383.51  22.29  0.000
Error   316  5437.56  5437.56   17.21
Total   319  6588.09


S = 4.14819   R-Sq = 17.46%   R-Sq(adj) = 16.68%


Unusual Observations for C5

Obs       C5     Fit  SE Fit  Residual  St Resid
  4  13.7000  5.0650  0.4638    8.6350      2.09 R
 20  28.2000  5.0650  0.4638   23.1350      5.61 R
 30  13.7000  5.0650  0.4638    8.6350      2.09 R
 33  28.2000  5.0650  0.4638   23.1350      5.61 R
 44  13.7000  5.0650  0.4638    8.6350      2.09 R
 48  28.2000  5.0650  0.4638   23.1350      5.61 R
 63  28.2000  5.0650  0.4638   23.1350      5.61 R
 67  13.7000  5.0650  0.4638    8.6350      2.09 R
```

```
100  20.7000  3.2560  0.4638   17.4440      4.23 R
120  20.7000  3.2560  0.4638   17.4440      4.23 R
128  20.7000  3.2560  0.4638   17.4440      4.23 R
143  20.7000  3.2560  0.4638   17.4440      4.23 R

R denotes an observation with a large standardized residual.


Least Squares Means for C5

C6            Mean  SE Mean
BA-R100    3.25600   0.4638
DART-R100  0.00638   0.4638
POP-R100   5.06500   0.4638
ST-R100    1.48950   0.4638


Tukey 95.0% Simultaneous Confidence Intervals
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-R100  subtracted from:

C6          Lower  Center   Upper  -----+---------+---------+---------+-
DART-R100  -4.933  -3.250  -1.566   (----*----)
POP-R100    0.125   1.809   3.493                (----*----)
ST-R100    -3.450  -1.767  -0.083       (----*----)
                                -----+---------+---------+---------+-
                                  -3.5       0.0       3.5       7.0


C6 = DART-R100  subtracted from:

C6          Lower  Center  Upper  -----+---------+---------+---------+-
POP-R100   3.3751   5.059  6.742                          (---*----)
ST-R100   -0.2004   1.483  3.167                (----*----)
                                -----+---------+---------+---------+-
                                  -3.5       0.0       3.5       7.0


C6 = POP-R100  subtracted from:

C6        Lower  Center   Upper  -----+---------+---------+---------+-
ST-R100  -5.259  -3.575  -1.892  (----*----)
                                -----+---------+---------+---------+-
                                  -3.5       0.0       3.5       7.0


Tukey Simultaneous Tests
Response Variable C5
All Pairwise Comparisons among Levels of C6
C6 = BA-R100  subtracted from:

           Difference       SE of           Adjusted
C6           of Means  Difference  T-Value   P-Value
DART-R100      -3.250      0.6559   -4.955    0.0000
POP-R100        1.809      0.6559    2.758    0.0297
ST-R100        -1.767      0.6559   -2.693    0.0356


C6 = DART-R100  subtracted from:

          Difference       SE of           Adjusted
C6          of Means  Difference  T-Value   P-Value
```

POP-R100	5.059	0.6559	7.713	0.0000
ST-R100	1.483	0.6559	2.261	0.1072

C6 = POP-R100 subtracted from:

C6	Difference of Means	SE of Difference	T-Value	Adjusted P-Value
ST-R100	-3.575	0.6559	-5.451	0.0000