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The use of megavoltage radiation therapy in the treatment of thymomas in rabbits: 19 cases

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Abstract

An overall median survival time (MST) and prognostic factors in rabbits with thymomas treated with megavoltage radiation therapy (RT) were determined in this multi-institutional retrospective case analysis. Medical records for 19 rabbits with suspected or confirmed thymomas treated with RT were evaluated for data including signalment, haematological and serum biochemistry abnormalities, presence of pleural effusion, radiation plan, body weight, total radiation dose and institution administering RT. Statistical significance of these factors related to overall survival was assessed. An overall MST for all 19 rabbits was 313 days; exclusion of 3 rabbits that died acutely during the first 14 days of RT yielded a MST of 727 days. The only factor associated with a significantly decreased survival time was having a body weight lower than mean body weight of 1.57 kg. Radiation treatment-associated complications were infrequent and included radiation-induced myocardial failure, radiation pneumonitis and alopecia.

Keywords
lagamorph, mediastinal mass, oncology, rabbit, radiation therapy, thymoma

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Introduction

Primary thymic neoplasia includes thymoma, a benign neoplasm of the thymic epithelial cells; thymic carcinoma, a malignant neoplasm of the epithelial thymic cells; and lymphoma, a malignant neoplasm of the lymphoid component of the thymus. Thymic carcinomas are rare and most arise *de novo*, although in human medicine there are individual reports of transformation of thymomas into thymic carcinomas.[1]

Thymomas have been previously reported to comprise 7% of neoplasms in 55 colony rabbits with a reported age range of 1–4 years.[2] Thymic carcinoma has also been reported in a rabbit.[3] In general, rabbit thymomas tend to be slow growing, potentially local invasive tumours that rarely metastasize.[4] Other neoplastic mediastinal

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histologies reported in dogs, cats and rabbits include ectopic thyroid or parathyroid tumours, aortic body or carotid body tumours, heart base tumours, lipomas, mast cell tumours and metastatic neoplasia affecting the mediastinal lymph nodes.[5] Non-neoplastic mediastinal changes reported in multiple species including rabbits include abscesses, thymic hyperplasia, thymic haemorrhage, mediastinal cysts and thymic amyloidosis.[6,7]

Clinical signs associated with a thymoma in rabbits include dyspnoea and tachypnea and are directly attributable to the presence of a space-occupying thoracic mass or secondary pleural effusion.[7] Bilateral exophthalmos has been reported secondary to thymoma and is because of the pooling of blood in the retrobulbar venous plexus as a consequence of cranial vena cava compression.[8] This vascular compression also can lead to oedema of the head, neck and forelimbs.[7] Thymomas in rabbits have also been reported as incidental findings, either on routine thoracic radiographs or on necropsy examination with no reported clinical signs attributable to a mediastinal mass.[4]

Suspected paraneoplastic syndromes in rabbits with thymomas include haemolytic anaemia[9] and dermatoses.[10] Paraneoplastic hypercalcaemia in a rabbit with thymoma has been reported but may represent a non-neoplastic high normal calcium as the reference range used was for canine patients.[11] Paraneoplastic syndromes commonly associated with thymomas in dogs such as myasthenia gravis have not been reported in rabbits. The pathophysiology of thymoma-induced paraneoplastic syndromes in rabbits has not been well characterized. The most commonly reported therapy for thymomas in dogs and cats is surgical cytoreduction with long-term survival reported for patients surviving the immediate postoperative period.[12] There are several case reports detailing the treatment of rabbit thymomas with surgery, radiation, surgery and radiation therapy (RT) and adjunctive chemotherapy with a wide range of individual survival times.[13–15]

To the authors' knowledge, there are no retrospective studies examining the role of RT in the treatment of rabbit thymomas; two case series previously reported the use of RT in the treatment of rabbit thymomas.[14,15] This study includes all five of the rabbits from these two previously published case series. The purpose of this study is to retrospectively evaluate the medical records of rabbits with suspected or confirmed thymomas, to determine clinical signs, prognostic factors, response to therapy and survival data for rabbits treated with RT. It is our hypothesis that RT will result in long survival times with minimal side effects.

Materials and methods

Patient population

The medical records of rabbits with thymoma treated with either coarsely or definitively fractionated RT from five tertiary and specialty veterinary medical institutions were reviewed. Definitive (radiation) therapy protocols were defined as four Gray (Gy) or less per fraction delivered on a Monday, Wednesday and Friday schedule or more frequently.[16] All other RT protocols were defined as coarsely fractionated. Participating institutions included Animal Medical Center, VCA Bay Area Veterinary Specialists, Cornell University, Tufts University and the University of California, Davis. Inclusion criteria were as follows: rabbits with cytologically or histologically confirmed or suspected thymomas treated with RT used either as a sole modality or as adjunctive therapy.

Procedures

Complete medical records from the Animal Medical Center and VCA Bay Area Veterinary Specialists were reviewed. Electronic medical records from University of California, Davis, were reviewed. A questionnaire was sent to the remaining institutions, Cornell University and Tufts University, and completed by a single author at each institution. All original biochemical analyses, cytology and histopathology results, if available, were reviewed. The decision to submit original medical records, electronic medical records or the questionnaire was based on the availability of the original medical records for review, the policies of the institution in terms of releasing medical records and the resources available at the participating institution to compile the information. The data extracted from either

the original medical record or from a completed questionnaire about each case included results of the history, physical examination and biochemical analyses including complete blood counts (CBC), serum biochemistry panels and urinalysis; cytology and/or histopathological characterization of the mediastinal mass; interpretation of imaging studies including thoracic radiographs, computed tomography (CT) scans and thoracic ultrasound scans; RT treatment prescriptions and RT plans; the use of prednisone or chemotherapy; the use of surgery as an adjunct therapy; and long-term case outcome including potential radiation side effects, tumour recurrence, cause of death, date of death or date of last contact. Available necropsy results were evaluated. Anaesthetic protocols were reviewed, but were not available for the majority of cases because of lack of access to the original medical records. Anaesthetic protocols varied between cases and included a variety of premedications, injectable and inhalation anaesthetic agents.

Survival times were calculated using the Kaplan–Meier method. An overall survival time was calculated from the day of first RT treatment to the end event, defined as death because of any cause. Rabbits lost to follow-up were censored on the last day of contact. Disease-free intervals were not calculated, because the date of resolution of clinical signs was not known for most rabbits. Univariate analysis using a log-rank test was used to assess the following variables for effect on overall survival time: sex, institution, breed, weight above or below median weight, weight above or below mean weight, presence of any presenting signs, presence of tachypnea or exophthalmos or lethargy as initial clinical sign, presence of pleural effusion, presence of anaemia, presence of hypercalcaemia, presence of lymphocytosis, therapy with prednisone, therapy with other chemotherapeutic agents, total RT dose above or below mean RT dose and the use of either definitively or coarsely fractionated RT protocols. All statistical analyses were performed using a commercially available statistical software program (Stata 10.0, StataCorp, LP College Station, TX, USA). For all analysis, a P value of <0.05 was considered statistically significant.

Results

Patients

Twenty-seven rabbits with mediastinal masses were identified for this study. Rabbits (7) were excluded because they did not receive RT and 1 rabbit was excluded because at necropsy, the mediastinal mass was determined to be mediastinal lymphoma. Nineteen rabbits met the inclusion criteria. The time period of the study defined as the first date of RT treatment was November 2003 to April 2010. The median age at diagnosis was 6.7 years (range 3.2–10.0 years). Breeds represented included Netherland dwarf ($n = 12$), mixed or unknown breed ($n = 4$), angora ($n = 1$), silver martin ($n = 1$) and rex ($n = 1$). Sex distribution included male intact ($n = 1$), male castrated ($n = 8$) and female spayed ($n = 10$). None of the animals in this study population were intact females. Median weight at admission was 1.45 kg and mean weight at admission was 1.57 kg (range 0.99–2.8 kg, SD 0.51).

Clinical signs before diagnosis and RT included: bilateral exophthalmos ($n = 13$), respiratory changes including tachypnea, increased respiratory effort or respiratory distress ($n = 12$) and lethargy ($n = 2$). Rabbits with no clinical signs attributable to thymoma comprised a minority of the study population ($n = 2$). Bilateral exophthalmos, rather than respiratory signs, was the most common clinical sign associated with thymomas in this study.

Diagnostic blood parameters or clinician commentary about these results including CBC ($n = 13$) and serum biochemistry results ($n = 14$) were available for the majority of rabbits. Initial CBC results were normal for eight rabbits. CBC abnormalities were noted in five rabbits and included anaemia ($n = 2$; hematocrit (HCT) 30% with normal range of 35–50%, and HCT 29.9% with normal range of 35–50%) and mature lymphocytosis ($n = 2$; 9129 μL^{-1} with normal range of 1500–7000 μL^{-1} and 18 564 μL^{-1} with normal range of 1200–7000 μL^{-1}) and in a single rabbit ($n = 1$), monocytosis (1152 μL^{-1} with normal range of 0–300 μL^{-1}), eosinophilia (288 μL^{-1} with normal range of 0–100 μL^{-1}) and basophilia (720 μL^{-1} with normal range of 0–500 μL^{-1}).

Serum biochemistry panels were normal for four rabbits. Serum biochemistry abnormalities included: elevated liver enzymes, either alanine transaminase ($n = 3$; 72 IU L^{-1} with normal range of 48–70 IU L^{-1}; 82 IU L^{-1} with normal range of 10–45 and 62 IU L^{-1} with normal range of 10–45), alkaline phosphatase ($n = 2$; 39IU L^{-1} with normal range of 4–20IU L^{-1} and individual result not available in one rabbit) or both ($n = 1$, results not available); hyperglycaemia ($n = 3$; 188 mg dL^{-1} with normal range of 80–150 mg dL^{-1}; 221 mg dL^{-1} with normal range of 75–145 mg dL^{-1} and 212 mg dL^{-1} with normal range of 80–150 mg dL^{-1}); elevated creatine kinase ($n = 3$; 7350 IU L^{-1} with normal range of 140–372 IU L^{-1}; 3519 IU L^{-1} with normal range of 140–372 IU L^{-1} and 3117 IU L^{-1} with normal range of 140–372 IU L^{-1}); elevated albumin ($n = 2$; 3.9 mg dL^{-1} with normal range of 2.7–3.6 g dL^{-1} and 5.3 mg dL^{-1} with normal range of 2.4–4.5 g dL^{-1}); elevated blood urea nitrogen ($n = 1$; 31 mg dL^{-1} with normal range of 17–24 mg dL^{-1}); elevated globulins ($n = 1$; 4.0 g dL^{-1} with normal range of 1.5–2.8 g dL^{-1}) and decreased phosphorous ($n = 4$; 3.1 mg dL^{-1} with normal range of 4.4–7.2 mg dL^{-1}; 3.5 mg dL^{-1} with normal range of 4.0–6.2 mg dL^{-1}; 3.2 mg dL^{-1} with normal range of 4–6.9 mg dL^{-1} and 4.3 mg dL^{-1} with normal range of 4.4–7.2 mg dL^{-1}). Elevated total calcium was documented in five cases and presented in Table 1.

Imaging of the mediastinum was performed in all rabbits using one or more of the following: thoracic radiographs ($n = 17$), thoracic ultrasound ($n = 12$) and thoracic CT scans with pre and postcontrast imaging ($n = 9$). Radiographic findings included a cranial mediastinal mass effect in all rabbits. Pleural effusion was observed in six rabbits. Thoracic ultrasound was used in 11 rabbits to obtain fine needle aspirates for cytology, and ultrasound reports were available for review in 10 rabbits. The mediastinal mass was described as cystic in 6 rabbits (Fig. 1) and noncystic in 4 rabbits. CT scans were performed in nine rabbits and were used to generate computerized RT treatment plans using the following treatment planning systems: Pinnacle3 (Milpitas, CA, USA) or Eclipse (version 8, Varian Medical Systems, Palo Alto, CA, USA). In 6 rabbits, a second CT was performed during RT (after the fourth of 10 treatments for 1 rabbit, after the fifth of 12 treatments for 2 rabbits, after the sixth of 10 planned treatments for 1 rabbit, after the seventh of 12 treatments for 1 rabbit and after the eighth of 10 treatments for 1 rabbit). A new RT plan was developed using the second CT to reduce radiation dose to neighbouring normal tissues. The six rabbits imaged with a second CT during radiation treatment showed a reduction in the mass volume ranging from 30.0 to 86.6% compared with the original CT scan. A single rabbit had a follow-up CT scan 11 months after the conclusion of RT showing an increase in the thymoma size from 7.1 (size of mass on CT scan performed after treatment 8/10) to 10.09 cm^3. The pretreatment tumour volume in this rabbit was 18.5 cm^3.

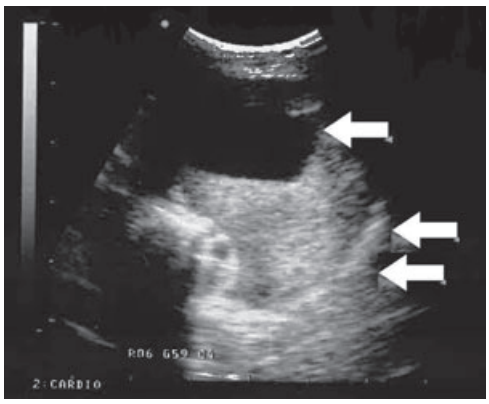

Figure 1. Ultrasound image of a thymoma from one of this study rabbits in lateral recumbency. Cystic (denoted with single arrow) and noncystic (denoted with double arrow) portions of the mass are visible. Ultrasound image courtesy of Dr Mark Matteucci, Bay Area Veterinary Imaging.

Table 1. Calcium levels in study population

Institution	Rabbit identification number	Calcium level (mg dL^{-1})	Institution calcium reference range (mg dL^{-1})
Animal Medical Center	AMC 2	16.2	8–15.5
VCA BAVS	BAVS 1	13.2	5.6–12.0
Tufts University	Tufts 2	15.2	9.6–14.8
Tufts University	Tufts 3	16.1	9.6–14.8
University of California, Davis	Davis 1	17.0	12–16

Characterization of mediastinal mass lesions was performed by cytology alone in 17 of 19 rabbits. A single rabbit had a mediastinal mass biopsy with no cytology performed and another rabbit had both cytology and a biopsy performed. Cytology was considered definitive of thymoma in 8 rabbits and suggestive of thymoma in 9 rabbits. All cytology results were generated by a board-certified clinical pathologist either at the treating institution or a referral laboratory.

Treatment

All rabbits were treated with RT while two rabbits had surgery following RT for additional cytoreduction. The exact rationale for the postradiation surgery was not readily apparent from the record and was carried out at the managing clinician's discretion. Six rabbits were treated with definitively fractionated protocols, whereas 13 rabbits were treated with coarsely fractionated protocols. All 6 of the rabbits treated with definitively fractionated RT protocols and three rabbits treated with a coarsely fractionated RT protocol had computer generated RT plans, whereas 10 of the rabbits treated with coarsely fractionated RT were treated with manually planned RT. A single rabbit was treated twice with coarsely fractionated RT 2 years apart, with the second RT treatment initiated because of presumed thymoma recurrence based on physical exam showing recurrent bilateral exophthalmos and thoracic radiographs showing a mediastinal mass. Total radiation doses for rabbits completing definitively fractionated protocols ranged from 42 to 48 Gy. The total radiation dose for rabbits completing coarsely fractionated RT ranged from 24 to 32 Gy. The total radiation doses for all rabbits completing either definitively or coarsely fractionated RT ranged from 24 to 48 Gy (median 32.4 Gy). All treatment plans were developed and approved by a board-certified veterinary radiation oncologist.

Additional information regarding definitive RT treatment plans was available for review in all cases. Initial radiation doses were delivered to rabbits in sternal recumbency in 4 of 6 cases with left and right oblique fields using wedges, allowing a more homogeneous dose to be delivered. The initial sternal positioning of the rabbit was chosen because it reduced respiratory distress compared with other positions. Treatment field positions were changed to lateral parallel opposed fields in these four cases as the tumour size decreased to reduce radiation dose to the surrounding tissue, particularly the heart and lungs. Initial radiation doses were delivered to rabbits in lateral recumbency in two cases. An example of a CT image acquired for radiation treatment planning from a rabbit treated with a definitive RT protocol in this study is shown in Fig. 2. A repeat CT image acquired from this same rabbit after treatment with 24.5 Gy is shown in Fig. 3. Positioning of the rabbits for CT scanning and RT was accomplished using conformable, repositioning devices such as Vak-Lok (MedTec, Orange City, IA, USA) or similar conformable positioning devices. A single rabbit was treated with intensity-modulated RT (IMRT). This rabbit was positioned in a customized mold support made of polystyrene beads coated in a moisture-cured polyurethane resin (MoldCare Pillow, Bionix Development Corporation, Toledo, OH, USA) for imaging and treatment. Before each treatment, orthogonal setup films were taking using an electronic portal imaging device and software

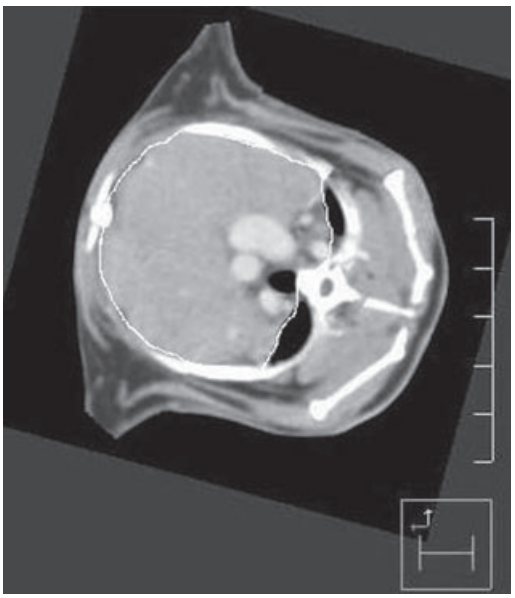

Figure 2. Transverse, pretreatment CT image of the mediastinum from one of this study rabbits treated with definitive RT; rabbit is in right-lateral recumbency. Line outlining tumour is shown. Image courtesy of Dr John Farrelly, Animal Medical Center, Pinnacle imaging system.

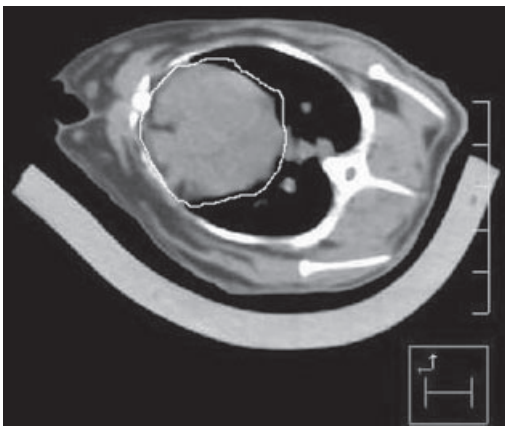

Figure 3. Transverse, CT image of the mediastinum from the rabbit in right-lateral recumbency shown in Figure 5, taken after treatment with 24.5 Gy. Lines outlining decreased tumour size is shown. Image courtesy of Dr John Farrelly, Animal Medical Center, Pinnacle imaging system.

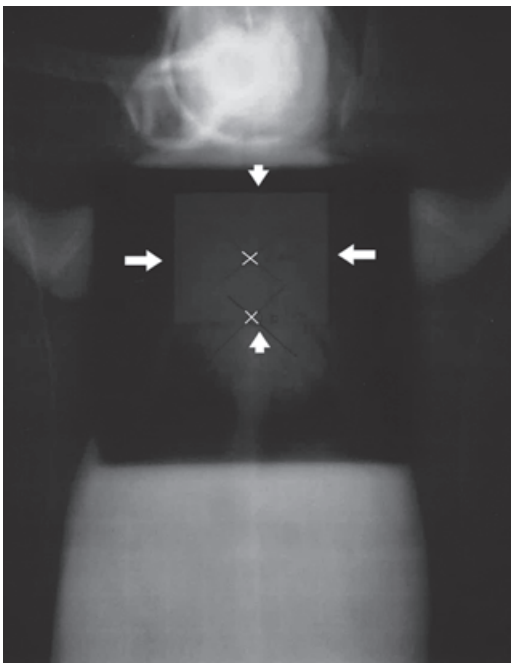

Figure 4. Port film of the thorax from one of this study rabbits treated with coarsely fractionated RT; rabbit is in ventrodorsal recumbency. Radiation treatment field of 5.5 × 5.0 cm is outlined with arrows. There are two isocentres marked with X. Caudally, the initial isocentre taken on a scout simulation film is noted and cranially, the adjusted isocentre after treatment field was determined by treating veterinarian. Blocks were not used as extent of disease precluded that option.

to verify positioning (Portal Vision Treatment Acquisition software version 7.3, Varian Medical Systems, Palo Alto, CA, USA). The IMRT plan was tested using IMSure QA software (version 1.22, Prodigm, Chico, CA, USA) as well as film and chamber measurements taken in virtual water.

The rabbits treated with coarsely fractionated plans were generally treated in either sternal or lateral recumbency using parallel opposed fields in the lateral or dorsal ventral directions, respectively. Information regarding the use of port films during therapy is available for three rabbits. In these cases, initial treatment port films were taken just before the first and subsequent port films were taken either the second- or third-treatment session. The second-port film was used to ensure accurate rabbit positioning, to visualize the mediastinum and to modify the radiation field size to reduce radiation exposure to normal surrounding tissues. The rabbits were often marked with ink to provide external anatomic landmarks to use for consistent radiation field alignment and placement. An example of an initial-port film from a hand-planned radiation treatment plan is shown in Fig. 4. Positioning during RT in these cases was provided using positioning devices similar to those used in the cases receiving definitive therapy.

Other therapies employed in these rabbits included surgery at 6 and 9 months post-RT ($n = 2$), prednisone as the only systemic therapy ($n = 5$) and chemotherapy ($n = 1$). Interestingly, the only rabbit treated with systemic chemotherapy (cyclophosphamide 50 mg m^{-2} every 4 weeks for seven treatments until death) presumably died from cyclophosphamide-induced renal failure. Histopathological results on necropsy examination confirmed that 75% of normal renal parenchyma was replaced with fibrotic connective tissue in the absence of any signs of bacterial infection. Histopathology of thymic tissue showed no evidence of disease.

Outcome

Clinical response to therapy and disease-free intervals was not available for all rabbits and was not calculated using statistical methods. The records for seven rabbits documented the dates of resolution of clinical signs. The time from the first RT treatment to resolution of clinical signs ranged from 4 to 42 days. Serial thoracic radiographs were taken of

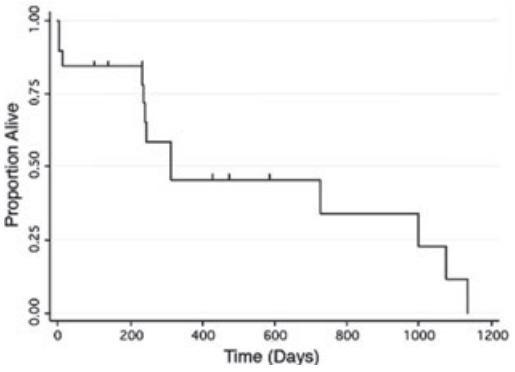

Figure 5. Kaplan–Meier graph showing overall survival of rabbits with thymoma treated with RT, including those cases in which death occurred during treatment period ($n = 19$). The MST was 313 days (95% CI 237–1000 days). Censored cases are indicated by tick marks.

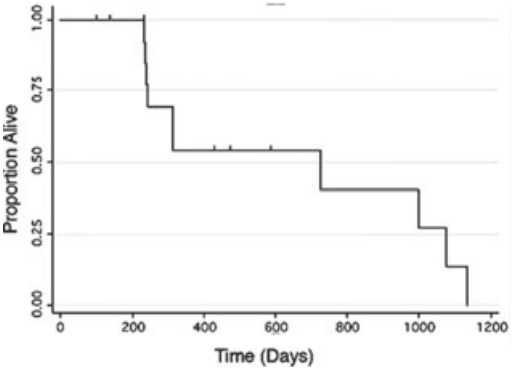

Figure 6. Kaplan–Meier curve showing survival for all rabbits completing radiotherapy ($n = 16$). MST was 727 days (95% CI 240–1077 days). Censored cases are indicated by tick marks.

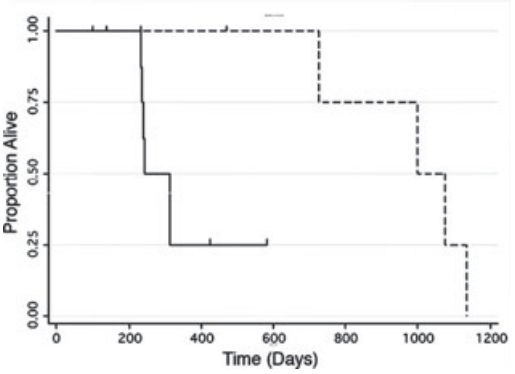

Figure 7. Kaplan–Meier graph showing survival of all rabbits living beyond the treatment period, with body weight either below (solid line) or above (dashed line) a mean body weight of 1.57 kg. This difference was statistically significant ($P = 0.02$). Censored cases are indicated by tick marks.

two rabbits to assess changes in thymoma size post-RT. In one rabbit, there was resolution of the clinical signs during RT, and decrease but not complete resolution of the size of the cranial mediastinal mass on follow-up radiographs. Subsequent follow-up thoracic radiographs at 6 months showed the size of the cranial mediastinal mass to be static. In the second rabbit, there was resolution of clinical signs after RT, but no appreciable change in the size of the cranial mediastinal mass was found on radiographs taken 6 months post-RT. An overall median survival time (MST) of 313 days was calculated for all of the rabbits [95% confidence interval (CI) 237–1000 days] (Fig. 5). An overall MST of 727 days (95% CI 240–1077 days) was obtained when excluding the three rabbits that died acutely during the course of RT (Fig. 6). The only variable found to significantly impact overall survival was body weight below the mean weight at admission ($P = 0.007$). Rabbits weighing less than 1.57 kg had a MST of 312 days, whereas rabbits weighing more than 1.57 kg had a MST of 727 days (Fig. 7). Body weight below or above the median weight of 1.45 kg at admission was not found to be a significant prognostic factor for survival ($P = 0.06$). This also proved true if the three cases that died during the course of RT were excluded with rabbits weighing less than the mean weight having a significantly shorter survival ($P = 0.02$). This variable was not statistically significant if the median weight was used ($P = 0.06$). Other tested variables were not found to be significantly associated with overall survival included and are presented in Table 2.

At the time of data collection, 14 rabbits had died and 5 were still alive. In six rabbits, the cause of death was not determined or available. For 3 rabbits, death occurred during the first 2 weeks of RT (definitively fractionated RT, $n = 1$ and coarsely fractionated RT, $n = 2$) with all 3 dying after extubation on the day of RT. None of these rabbits had necropsy examinations performed. Three other rabbits likely died, at least in part, because of recurrence of the thymoma. One of these rabbits had radiographic and ultrasonographic evidence of pleural effusion and a mediastinal mass six- and one-half months after completing a coarsely fractionated RT protocol. The second rabbit had

Table 2. Characteristics of study population and their significance

Characteristics	Number of rabbits affected (%)	*P* value of all rabbits	*P* value of rabbits completing RT
Sex		0.88	0.67
Male intact	1 (5.26)		
Male castrated	8 (42.11)		
Female spayed	10 (52.63)		
Weight below mean weight of 1.57 kg		0.007	0.02
Weight below median weight of 1.45 kg		0.58	0.06
Breed		0.32	0.30
Institution administering RT		0.68	0.74
Presence of any clinical signs at diagnosis	17 (89.47)	0.46	0.22
Present at diagnosis			
Exophthalmos	13 (68.42)	0.43	0.38
Lethargy	2 (10.53)	0.96	0.54
Tachypnea or dyspnoea	12 (63.16)	0.76	0.66
Pleural effusion	1 (35.29)	0.30	0.69
Anaemia	2 (22.22)	0.58	0.33
Lymphocytosis	2 (31.38)	0.46	0.50
Hypercalcaemia	5 (26.32)	0.90	0.61
Cytology definitive for thymoma	8 (42.11)	0.70	0.63
Treatment with prednisone	5 (26.32)	0.20	0.11
Treatment with chemotherapy	1 (5.26)	0.67	1.0
Definitive versus palliative RT protocol		0.71	0.65
Definitive RT	6(31.6)		
Palliative RT	13 (68.4)		
Total RT dose above or below mean dose		0.19	0.53
Surgical therapy	2 (10.53)	Not calculated	Not calculated

presumed recurrent thymoma 2 years after the first course of RT based on thoracic radiographs showing a mediastinal mass and recurrent bilateral exophthalmos and was subsequently treated with a single 8 Gy fraction. The rabbit was euthanized 8 months after the last RT treatment, without a necropsy. The third rabbit had histopathologically confirmed persistent thymoma tissue, pleural effusion and multiple brain granulomas secondary to *Encephalitozoon cuniculi* at necropsy. The remaining deceased rabbits died from renal fibrosis ($n = 1229$ days), radiation-induced cardiac failure ($n = 1240$ days) and liver failure ($n = 1727$ days).

Treatment toxicities

Complications related to therapy were documented in three rabbits. Presumed radiation-induced pneumonitis developed approximately 3 months after the conclusion of coarsely fractionated RT in one rabbit based on thoracic radiographs and clinical signs. On the basis of the RT plan, the minimum dose to the lung was 0.62 Gy, whereas the mean and maximum doses were 6.9 and 29.4 Gy, respectively. Therapy included prednisone, which had already been started as therapy for the thymoma, for the lower respiratory signs which persisted for the remainder of the rabbit's life (24 months). Death in this case was presumed to be due to both chronic respiratory disease and liver failure, although no necropsy was performed. The liver parenchyma of this rabbit was not in the radiation field. Radiation-induced myocardial failure developed 12 months after the conclusion of definitively fractionated IMRT in one rabbit, with severe multifocal myodegeneration and nonsuppurative myocarditis found histopathologically. On the basis of the RT plan, the minimum, mean and maximum dose to the heart in this rabbit were 3.8, 25.2 and 50.4 Gy, respectively. Suspected radiation-induced mild-to-severe hair loss was documented in one rabbit with alopecia limited to the RT field noted after receiving coarsely fractionated RT treatment. A single rabbit treated with coarsely fractionated RT and cyclophosphamide had histopathological evidence of significant bilateral renal fibrosis, and death because of chronic renal failure 7 months after the

start of RT. The renal changes were attributed to the chronic administration of cyclophosphamide.

Discussion

The results of this retrospective study demonstrate that megavoltage irradiation, either coarsely or definitively fractionated, is an effective treatment for rabbit thymomas, with long-term survival after therapy. Resolution of clinical signs, when observed, was rapid in both the definitively and coarsely fractionated RT groups and occurred within a range of 1–5 weeks after starting RT treatment. In the six rabbits with follow-up CT scans taken during therapy, decreases in tumour volumes ranging from 30.0 to 86.6% occurred, indicating that this tumour is radioresponsive. RT complications affected a minority of treated rabbits and included acute radiation field alopecia, delayed acute radiation-induced pneumonitis and late-term side effects of radiation-induced myocardial fibrosis.

Both coarsely and definitively fractionated protocols were used in this population of rabbits and the type of radiation protocol was not significantly associated with survival time in this study group. Coarsely fractionated protocols, whether based on manually calculated RT plans or CT-based computer generated treatment plan, include fewer anaesthetic episodes and radiotherapy sessions compared with definitively fractionated RT protocols and therefore involve less cost to the client and less anaesthetic risk to the rabbit. However, the risk of late-term side effects increases as the radiation dose per fraction increases.[17] The use of definitively fractionated protocols may reduce, but does not eliminate, the risk of radiation-induced side effects because of the close proximity of the thymoma to radiation sensitive tissue, such as the heart and lungs. This was illustrated by a rabbit in this study treated with a definitively fractionated IMRT plan, developing radiation-induced myocardial fibrosis; the mean dose to the myocardium in this case was 52.6% of the total dose to the thymoma. The rabbit that developed radiation pneumonitis was treated with a coarsely fractionated protocol. The occurrence of late-term side effects in this study was not clearly associated with a particular RT protocol. As regular follow-up and necropsy examinations were not completed in the majority of cases, it is likely that late radiation side effects were underestimated in this study. Although this study does not show a statistically significant difference in outcomes or radiation-related toxicity between the finely and coarsely fractionated patient groups, given the potential long-term survival of rabbits treated with megavoltage RT, the risks of late RT side effects associated with higher doses per fraction protocols should be discussed with owners.

The molecular aetiology of rabbit thymomas remains unknown; the cellular pathways involved in thymic cell transformation may provide therapy targets in the future, in addition to these therapies of radiation and surgery. Most thymomas are thought to arise from somatic cell transformation, although germ line changes associated with thymomas have been identified in humans.[18,19] Genetic changes observed in laboratory animal populations associated with thymoma formation include viral insertional mutagenesis causing the loss of tumour suppressor genes such as *p16INK4a* and *ARF*[20] and increased expression of oncogenes including c-myc.[21] In a study of feline neoplasia, thymomas were found to over express bcl-2 antiapoptotic proteins.[22] As with many neoplastic processes, the aetiology of thymomas in rabbits and other species is likely because of multiple genetic and epigenetic changes leading to thymic epithelial transformation.

A previous study reported an age range for rabbits with thymomas of 1–4 years, which was younger than this study population.[2] In that report, the study population consisted of rabbits with two or more neoplastic processes and therefore may have included rabbits with heritable genetic changes in tumour suppressor genes or proto-oncogenes, predisposing these rabbits to earlier onset neoplastic processes. The range of ages of rabbits in this study was older (3.2–10.0 years) and to our knowledge none of the rabbits in our study were diagnosed with more than one neoplastic process.

Initial diagnosis of mediastinal masses was often made using thoracic radiographs in this study. The normal mediastinum in rabbits can appear more radio-opaque on radiographs compared with the mediastinum of cats and dogs[23] as the

normal thymus in rabbits does not fully regress in adulthood.[24] Furthermore, thymic hyperplasia that is found in some rabbits can also cause significant thymic enlargement.[25] Ultrasound can be used to directly image the mediastinum and to obtain ultrasound-guided aspirates for cytology. Contrast enhanced CT imaging is considered superior to both plain radiographs and ultrasound exams in terms of imaging mediastinal masses for invasiveness and size in dogs and cats.[12] Staging systems and their prognostic value for thymomas in animal species have not yet been determined. Human thymoma patients are staged using the Masaoka Thymoma Staging System (stages I–IV) where higher stages are associated with progressive regional tissue invasion.[36]

Definitive diagnosis of thymoma can be difficult with cytology alone as is illustrated in our study population. Several factors make cytological diagnosis difficult, including variable amounts of lymphocytic proliferation in thymomas, better exfoliation of lymphocytes compared with the neoplastic epithelial cells, prevalence of thymic hyperplasia in normal adult rabbits[25] and the cystic nature of thymomas where the cytology of cystic fluid is often nondiagnostic in dogs and cats.[26,27] Ultrasound-guided fine needle aspiration of rabbit thymomas can yield predominantly small to intermediate non-neoplastic lymphocytes, predominantly neoplastic epithelial cells or a mixed population of both cell types. In contrast, mediastinal lymphoma in rabbits tends to be high grade and cytology yields intermediate to large-sized lymphocytes with characteristic cytological markers of malignancy.[28]

Methods for definitive diagnosis of mediastinal masses in dogs include histopathology on tissue samples obtained via ultrasound, CT guidance or thoracoscopy; immunocytochemistry on cytology samples or immunohistochemistry on biopsy samples; and clonality testing [Polymerase Chain Reaction for Antigen Receptor Rearrangement (PARR)] on the lymphocyte population.[29] Monoclonal antibodies for lagamorph epithelial neoplasms including cluster of differentiation 3 (CD 3) for T cells, CD 79a for B cells and cytokeratin for epithelial cells are available and are used to distinguish thymomas from other mediastinal lesions such as lymphoma. The use of flow cytometry to aid in the diagnosis of mediastinal masses in dogs showed a high specificity and sensitivity in distinguishing mediastinal thymoma from lymphoma.[29] A combination of cytology, flow cytometry, PARR and immunological staining may be necessary for definitive diagnosis in dogs and cats.[30] Currently, flow cytometry and PARR have not been validated for use in the diagnosis of rabbit thymomas (personal communication, Dr Anne Avery, 2010). A single rabbit in our study had an ultrasound-guided cutting needle biopsy to establish the diagnosis of thymoma and the remaining 18 rabbits were evaluated with aspiration cytology. None of the rabbit samples in our study had immunological staining or other advanced cytological diagnostics performed.

Reported therapies for thymomas in dogs, cats and rabbits include surgical removal, RT and surgery and adjunctive RT.[13–15,9,12,31,32] Surgical therapy in dogs and cats results in long-term survivals with the reported MST for dogs of 790 days and MST for cats of 1825 days in a recent retrospective study.[12] RT has been used as a sole modality in dogs and cats that are not surgical candidates or as an adjunctive therapy with a reported MST of 248 days in dogs and 720 days in cats.[31] RT has also been used as an adjunctive therapy postoperatively for incompletely resected thymomas in dogs and cats.[32] Two of the rabbits in this study had surgical cytoreduction after the completion of RT. This variable was not examined for effect on survival in this study because of the low number of rabbits treated with both radiation and surgery. This study shows that the median survival for rabbits with thymomas treated with RT is shorter than the reported median survival of dogs and cats with thymomas treated with surgery,[12] although because of the retrospective nature of these studies, direct comparisons cannot be made. This may support the concept that surgical therapy is the treatment of choice for thymomas in rabbits, as well as dogs and cats. However, surgical removal of rabbit thymomas is technically challenging.

No paraneoplastic syndromes were identified in this group of rabbits. Hypercalcaemia has been reported in rabbits diagnosed with thymoma.[13] Given the wide fluctuations in serum calcium in normal rabbits, the findings of hypercalcaemia

in the previous study may have been unrelated to the disease.[9,13,37] In this study, elevated total calcium was identified in 5 of 17 rabbits on initial serum biochemistry analysis; within this group normal phosphorous levels were observed in 2 rabbits and elevated phosphorous was observed in 1 rabbit which also had concurrent azotaemia. Low phosphorous levels were observed in two rabbits. Ionized calcium levels were not measured in any of the rabbits. Follow-up blood work was not consistently available for the hypercalcaemic rabbits after the start of therapy to determine if hypercalcaemia resolved with therapy. Serum calcium levels in normal rabbits are generally 30–50% higher compared with dogs and cats and the rabbit normal reference ranges for total calcium are reported at 13–15 mg dL^{-1} in Table 1, varying by institution. In rabbits, calcium is passively absorbed from the gastrointestinal tract based on the amount of dietary calcium ingested, rather than the metabolic needs of the rabbit and the excess is excreted in the urine as calcium carbonate. Therefore, postprandially, the calcium levels can rise significantly,[33] making it difficult to ascertain from these data if the observed hypercalcaemia was secondary to normal dietary intake or a paraneoplastic syndrome. Hypercalcaemia in one dog with a confirmed thymoma was shown to be secondary to an elevated Parathyroid hormone-related protein (PTHrp) level which after surgical resection declined rapidly along with resolution of the hypercalcaemia.[34] Hypercalcaemia was not identified as significantly associated with overall survival in this study.

Additional reported thymoma-related paraneoplastic syndromes in rabbits and cats include exfoliative dermatitis lesions affecting the face, pinnae, neck and dorsum.[35,11] In a previous study, histopathology of suspected paraneoplastic dermal lesions in a rabbit with a thymoma showed infiltration of the superficial dermis with lymphocytes, lymphocytic mural folliculitis and absence of sebaceous glands.[11] Thymoma-associated exfoliative dermatitis in cats shows a very similar infiltration of the superficial dermis with primarily CD 3+ T cells.[35] In both species, thymoma-induced immune dysregulation is thought to lead to T-cell recognition of skin self-antigens. Thymoma-related skin lesions were not identified in this study group.

The only significant prognostic variable found in this study was body weight less than the mean weight of 1.57 kg. This was observed on Kaplan–Meier analysis of all 19 rabbits and Kaplan–Meier analysis of the 16 rabbits that survived the first 2 weeks of RT. This finding could be because of physiological reasons such as anorexia or cancer cachexia because of increased tumour burden, poor body condition score because of concurrent non-neoplastic disease or type I error given the small number of overall cases. The observation that weight below the mean body weight of 1.57 kg was significantly associated with shorter survivals, whereas weight below the median of 1.45 kg was not suggests that this result may be because of an artefact in the data rather than a real finding. This finding would need to be evaluated in future studies to see if it remains a significant prognostic factor.

Limitations to this study include its retrospective nature, the lack of confirmatory biopsies in most rabbits to establish the definitive diagnosis of thymoma, the variable radiation prescriptions delivered to the rabbits, the variable radiation fractionation schedules, the lack of standardized follow-up leading to a lack of information on responses and recurrences for many of the rabbits and the lack of necropsies for most of the deceased rabbits. The majority of the rabbits were diagnosed based on a single cytological examination of a fine needle aspirate and then treated. Most rabbits did not have necropsies performed, therefore most did not have a confirmed histopathologic diagnosis of thymoma. It is possible that some of the rabbits had some other type of mediastinal disease such as thymic lymphoma. The response to therapy information was also not standardized, as one would expect in a retrospective study. Rabbits (7) had no information available as to the date of resolution of clinical signs, whereas only 6 rabbits had repeat CT imaging during RT and 2 rabbits had repeat thoracic radiographs following RT.

The results of this study demonstrate that RT, delivered in either a definitively or a coarsely fractionated protocol, is an effective treatment for rabbits with thymoma, resulting in an overall MST of 727 days for rabbits surviving the initial 2 weeks of RT. Prospective analysis using rabbits with confirmed thymomas randomly assigned to either

a standardized definitively or coarsely fractionated RT protocol with standardized follow-up including imaging to assess response to therapy, standardized recording of resolution of clinical signs, recording of time to thymoma recurrence and necropsy of all subjects to determine remission status at the time of death would be helpful to establish an ideal RT prescription, identify additional prognostic indicators and potential side effects not found in this study. Furthermore, the use of CT generated treatment plans for all rabbits in such a study would provide radiation dosing information for adjacent tissues such as lungs, heart and spinal cord and the development of late-term side effects in each group could be compared and evaluated thereby providing information and guidelines about the specific radiation tolerance of these tissues in this species.

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