THYMOMAS IN RABBITS

Rabbits possess a persistent thymus, which does not involute with age. The thymus is comprised of thymic epithelium, reticuloendothelial tissue, and lymphoid tissue. Primary thymic neoplasms recognized in rabbits include (1) benign neoplasm of thymic epithelial cells, (2) thymic carcinoma, a rare malignant neoplasm of the epithelial cells and (3) thymic lymphoma, which is a malignant neoplasm of the lymphoid tissue of the thymus. Clinically, thymomas are diagnosed as lymphoid-rich, epithelial-rich, or mixed lymphoepithelial. The incidence in pet rabbits is largely unknown, however in one retrospective study, thymomas comprised 7% of all neoplasms in 55 colony rabbits in rabbits 2-4 years of age in 1949. More recently, in a retrospective evaluation of 1,100 female rabbits more than 2 years of age submitted for necropsy over a 17-year period, 4 cases of the 234 rabbits that had neoplasia were thymomas (Andres 2012). Literary resources are mainly comprised of clinical case reports (Guzman, Kunzel, Pilny, Bennett, Kovalik, Wagner, Quesenberry, Florizoone, Vernau). Based on current literature, there is no specific sex predisposition, but the common age range is 3-10 years of age, with the median age of 6 years (Kunzel, Andres). Certain breeds have been consistently prominent in clinical case reports, including Netherland dwarves and Lionhead rabbits (Kunzel, Andres).

Clinical signs commonly include severe dyspnea and nasal flaring, third eyelid protrusion, orthopneic body position, and bilateral exophthalmos due to caval compression resulting in the pooling of blood in retrobulbar venous plexus. Owners may report a clinical history of exercise intolerance, inappetence, coughing, and hair loss. Hair loss may be attributable to the development of sebaceous adenitis, a common syndrome associated with thymoma as seen in cats, which is characterized on histology as lymphocytic mural folliculitis and sebaceous gland atrophy. On presentation, cardiac murmurs due to compression and pleural effusion may also be noted. Differentials for thymomas include mediastinal abscesses, mediastinal lymphadenopathy, heart base tumors, thymic hyperplasia, and thymic amyloidosis (Kunzel, Andres).

Clinical Approach & Diagnosis

Stabilization of the patient may be challenging however diagnostics can be acquired safely to diagnose thymoma and relieve the rabbit. Characterization of the patient’s oxygen responsiveness and degree of dyspnea is imperative. Rabbits have a small thorax and less thoracic respiratory volume compared to abdominal volume. They are obligate nasal breathers, however they use nasal twitches (rates of 0-150 per minute) for communication and sensory function and these rates differ and are separate from true respiratory rates (30-60 bpm). Nasal movements should not be counted to obtain a respiratory rate, as only thoracic movements truly qualify the rate. The diaphragm, rather than intercostal muscles, are used for respiration in rabbits at rest, therefore any
compression of the abdomen or presence of abdominal organomegaly can significantly compromise respiration. Signs of severe dyspnea can be easily missed and premature attempts to acquire diagnostics in these patients can result in compromise of stabilization efforts. Forceful nasal flaring and open-mouth breathing are poor prognostic signs. A T-fast scan of the thorax (with oxygen supplementation and gentle handling) can help characterize the thymic architecture, dimensions, associated effects on the heart and pleural space, and aid in thoracocentesis if needed to relieve clinical signs. Thoracocentesis in rabbits shares a similar approach to that performed in cats and dogs. Often, thoracic ultrasound confirms thymic enlargement and demonstrates if the structure is cystic, which may also require therapeutic aspiration to reduce dyspnea.

Safe aspiration of the pleural effusion and cyst can dramatically reduce clinical signs associated with dyspnea. The aspirates should be saved and submitted for cytology and culture, as this can aid in diagnosis. Fine needle aspirates of the mass and true-cut biopsies of anesthetized patients may not consistently provide a definitive diagnosis for the type of thymic neoplasia present. However, the presence of small mature and/or pleomorphic lymphocytes with prominent nuclei is clinically consistent with thymic lymphoma, which appears to be the most common form encountered in practice (Kunzel). Radiographs of the stable patient demonstrate the presence of a large soft tissue mass in the cranial mediastinum, often causing significant tracheal elevation and possible cardiac compression. Computed tomography can also be performed to characterize the mass. Complete blood counts in thymoma patients can be variable, but can include a leukocytosis (>10,000) with lymphocytes comprising more than 70% of the white blood cell differential (LL, Quesenberry, Andres). Often, this is indicative of thymic lymphoma. One study has retrospectively evaluated the cytological diagnoses in rabbits that underwent thoracotomy and/or necropsy for 13 confirmed cases (Kunzel). Five of 13 cases had lymphocyte-dominant thymomas, 1 case was epithelial dominant, 4 were mixed lymphoendothelial thymomas, and 2 were not determined. Pleural effusion was consistent for both lymphoid and epithelial-rich thymomas (Kunzel).

Management of Thymomas

Major treatment options include surgical removal via thoracotomy, radiation therapy, and adjunctive or sole palliative chemotherapy. Surgical removal is the treatment of choice for thymoma, which is curative with complete excision. Surgical removal, though reported and possible, runs a high risk of post-operative mortality. In the single study that has evaluated surgical survival, 5 of the 7 rabbits that underwent thoracotomy died within 3 days of surgery, and one survivor succumbed to thymoma recurrence 6 months post-operatively (Kunzel). Mean survival for the remaining rabbit was 955 days after surgery and was euthanized due to mass recurrence.

Several reports have evaluated the survival time in rabbits that have undergone radiation therapy, some in combination with chemotherapy. Currently, these studies reveal therapeutic successes with minimal RT–induced side effects and is associated with improved survival in clinical patients. Andres et al. examined the use of megavoltage RT as treatment for thymomas in a multi-institutional retrospective study that included VCA San Diego, VCA Bay, Tufts, UC Davies, and Cornell and Animal Medical center (NY). In this study, 19 rabbits met the inclusion criteria, 5 of which also received prednisone and 1 received cyclophosphamide concurrently. The major conclusions, while comparing definitive RT and palliative RT protocols, revealed that the overall median survival time for rabbits was 313 days. Thymomas were very radio-responsive, resulting in 30-86% reductions in tumor volume. Aside from anesthetic risk, RT-associated side effects

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were infrequent, but did include field alopecia, delayed acute RT-pneumonitis, and RT-induced myocardial fibrosis. The study did reveal one significant variable, body weight. For those that weighed less than 1.57 kgs, MST was 727 days and 312 days for those that weighed more than 1.57 kgs.

In one recent study, the use of volumetric modulated arc radiotherapy (VMAT) was used to definitively treat 15 cases. Six fractions of 6.67 Gy over an 11-day period was planned with rabbits to be treated on days 1, 3, 5, 7, 9, and 11 (Doler). No necropsies were performed, however organ at risk values were calculated demonstrating reduced radiation exposure with the use of radiation planning. MST was 777 days, and reduction in tumor sizes by 30-40% were achieved with some reduction in clinical signs after the first dose, and a complete response was still noted 6 months after treatment. Average anesthesia time for the treatments were not noted, however general anesthesia was maintained via facemask, and the authors of this study noted that injury due to intubation could have affected survival in the compared Andres study.

Commonly, palliative chemotherapy is elected and this can provide survival times that mirror survival times reported in the aforementioned RT studies. Protocols commonly employ prednisolone, L-Asparaginase, and cyclophosphamide, and vincristine. Other protocols can include doxorubicin (Quesenberry). The authors have had 2 cases achieve survivals of 1,045 days and 663 days with daily prednisolone treatment, two to three L’ Asparaginase treatments SQ, and single or two doses of oral cyclophosphamide. The authors have employed doses much lower than those reported for cats and dogs, however this has resulted in less severe immunosuppression. Repeat CBCs are recommended within 7 days after implementing treatment for L-Asparaginase, with concurrently implementation of prednisolone. If L-Asparaginase doses are repeated, patients should be pre-treated with diphenhydramine 1-2mg/kg SQ prior to repeat administration. After a cyclophosphamide dose has been administered, a repeat CBC should be performed every 7 days for 2 weeks to monitor for heteropenia.

Sebaceous adenitis has been successfully managed with cyclosporine based on few clinical case reports (Jassies, Kovalik), however carries the risk of additional immunosuppression. Often, the additional use of immunosuppressives should be cautiously used, as the medications themselves can cause renal and liver disease. This can also cause suppression of those compensatory immune responses that prevent *Encephalitozoon cuniculi* migration in infected rabbits. We have treated rabbits successfully with topical phytosphingosine salicylol (0.05%) microemulsion spays, and have demonstrated resolution of the disease using the Rabbit Dermatitis Extent and Severity Index (Kovalik).

In conclusion, thymoma’s should be considered as a differential in rabbits greater than 3 years of age presenting with respiratory distress. Treatments are available, thymic lymphoma appears to be very responsive to RT and/or palliative chemotherapy. These treatments can help reduce clinical signs and provide a good quality of life in animals with survival times that may range from 1-3 years.

FERRET LYMPHOMA
Lymphoma connotes a solid-tissue tumor composed of neoplastic lymphocytes in visceral organs, skin, or lymph nodes throughout the body (Antinoff). To date, lymphoma is the most common malignant neoplasia reported in the domestic ferret at 10-15% of all
neoplastic presentations in the US and Europe, and lymphoma is the third most common neoplasia of ferrets, behind adrenocortical neoplasia and insulinoma. It is documented as a spontaneous neoplasia (Mayer, Quesenberry), however there have been reports of horizontal transmission via cell and cell-free inoculation (Erdman), which suggests that there may be a viral etiology, however an agent has never been reported. There is one report of Helicobacter mustelidae-associated (MALT) gastric B-cell lymphoma (Erdman), and this syndrome appears to mimic gastric B cell lymphoma caused H. pylori in humans.

Ferret lymphoma can occur across a number of age groups and has no specific sex predilections. In the early literature describing the disease, ferret lymphoma was classified by age of onset and assigned distinct prognosis, i.e. the aggressive and quickly fatal juvenile onset lymphoma form and the adult chronic onset form. This generalized classification scheme has been since retracted due to new clinical reports that reveal there is no specific age and cell-type trend. Most resources characterize lymphoma by cell line, i.e. large cell, lymphoblastic lymphoma (T cell) or small cell, lymphocytic lymphoma (B cell). Finally, there are several studies that report disease based on location, which include but are not limited to multicentric lymphoma (Ferreira), cutaneous lymphoma (Xi, Rosenbaum), malignant B-cell lymphoma with Mott cell differentiation (Gupta), polyostotic lymphoma (Long), epitheliotropic gastrointestinal T-cell lymphoma (Sinclair), focal thoracolumbar spinal cord lymphoma (Ingrao), myelo-osteolytic plasmacytic lymphoma in the femur (Eshar), and gastrointestinal lymphoma (Lee).

Due to the substantial variation in lymphoma classification in ferrets, there has been a call for an adoption of the standardized classification system for ferret (Mayer). Currently, most clinicians develop diagnostic plans to (1) stage, (2) grade, and when possible, (3) phenotype lymphoma in clinical patients. Until a universal classification scheme can be established for ferret lymphoma, most pathologists and oncologists characterize lymphoma based on the World Health Organization (WHO) staging system. Staging identifies the anatomic location of the neoplasia and the measure of dissemination throughout the body. A 5 level staging scheme (Table 1) has been adapted from Antinoff and Mayer. Cell morphology characterization, or grading, is also imperative when classifying lymphoma type and qualifies prognosis (Table 2). In clinical practice, large cell versus small cell, round versus irregular, and nuclear size are used to classify cell type and tumor behavior from tissue aspirates.

<table>
<thead>
<tr>
<th>Table 1. Anatomic Location of Lymphoma</th>
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<tr>
<td>Staging</td>
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<tr>
<td>---------</td>
</tr>
<tr>
<td>Multicentric</td>
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<tr>
<td>Alimentary</td>
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<td>Mediastinal</td>
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Extranodal: Renal, CNS, Ocular, Cardiac

Cutaneous: Severe skin ulceration with or without nodular skin masses, usually seen along the ferret, sacral area, inguinal area, extremities

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<tr>
<th>Nuclear Size (relative to [RBC] Size)</th>
<th>Small: ≤ 1 RBC</th>
<th>Medium: &gt;1 but &lt; 3 RBC</th>
<th>Large: ≥ 3 RBC</th>
</tr>
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<tbody>
<tr>
<td>Mitotic Index</td>
<td>Low: &lt; 3</td>
<td>Intermediate: 3-8</td>
<td>High: &gt; 8</td>
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<tr>
<td>Other Descriptives</td>
<td>Nuclear morphology</td>
<td>Nucleoli</td>
<td>Distinct</td>
</tr>
<tr>
<td></td>
<td>Round</td>
<td>Indented/asymmetric/irregular</td>
<td>Indistinct</td>
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Phenotyping provides vital prognostic information and a definitive diagnosis. If tissue can be acquired, immunohistochemical stains help define the cell line as B or T cell. CD3 is a T cell marker, and CD 79α is a B cell marker. As in feline and canine medicine, the prognosis is based on cell line characterization, which predicts the progression of the disease process and response to treatment. As flow cytometry becomes more widely available for ferrets (Music), this may also be used to help define cell lines based on blood sample acquisition alone.

Clinical Signs, Physical Examination and Diagnostics

There is no universal clinical presentation for ferret lymphoma, and in some cases ferrets are asymptomatic. Clinical signs are nonspecific, but can include lethargy, anorexia, weight loss, ataxia, weakness, diarrhea, dyspnea, and respiratory signs (Suran, Antinoff, Mayer). Gastrointestinal signs are common, however this should not be confused with chronic gastrointestinal disease. Mesenteric and peripheral lymphadenopathies can be present with other disease processes, this clinical finding is not pathognomonic for lymphoma, however, intra-abdominal lymphadenopathy occurs with multicentric lymphoma in ferrets.

The diagnostic approach for ferrets with suspected lymphoma should include a minimum database (CBC, biochemistry screen, urinalysis), diagnostic imaging (radiographs, ultrasound, CT or MRI), and cytology obtained from aspirates, however biopsy tissue is preferred. The most consistent CBC finding in ferrets with lymphoma is a nonregenerative anemia. (Ammerbach). Lymphocytosis and thrombocytopenia are rare. If a lymphocytosis is present, with total white blood cell counts exceeding 30,000, a true lymphocytic or lymphoblastic leukemia may be present (Mayer). Hyperglobulinemia has
rarely been reported in ferrets with multiple myeloma, however a serum or urine protein
electrophoresis may help characterize if a monoclonal gammopathy is present (Eshar). It
is important to note that the presence of a hyperglobulinemia is also a hallmark of
ferrets that suffer from Aleutian’s disease, which is a viral condition that also causes a
hyperglobulinemia (Hess). In some cases, biochemical analysis reveal elevation in liver
and renal enzymes if disease is causing organ function compromise, but these findings
are not specific to lymphoma. One report of a hypercalcemia as a paraneoplastic
syndrome of lymphoma has been reported (Fisher), however it is rare.

Cytology from organ or lymph node aspirates, as seen in humans, show poor correlation
to definitive histologic diagnosis based on WHO classification, however it can help
provide the bases for tissue sampling based on cytological features (Antinoff, Hehn).
Cytologic hallmarks for lymphoma are a monomorphic population of lymphocytes and
the absence of peripheral blood elements (Antinoff). Evaluation of these samples by
experienced pathologists is imperative, as false positives due to misdiagnosis in ferrets
with reactive lymph nodes can occur. Once study revealed that the lymph node cell
distribution in normal ferrets includes 50-60 small lymphocytes, 2-3 lymphoblasts and
promylocytes, and 0-1 macrophages, plasma cells and nondegenerate neutrophils per
200 x field (Paul-Murphy). Biopsies are strongly recommended for a definitive diagnosis.
Gastric biopsies have been described. Scapular and popliteal lymph node biopsies can
be easily obtained with little surgical complication in ferrets. Patients that present with
bone lesions should undergo bone marrow aspiration or careful bone biopsy to further
characterize aggressive ostotic disease.

Diagnostic imaging can help aid in staging and collecting samples for evaluation. In a
recent study evaluating the image findings in ferrets with lymphoma, Suran et al.
concluded that the most common imaging finding was intra-abdominal lymphadenopathy
and mild peritoneal effusion in ferrets with multicentric lymphoma, which is the most
commonly reported in ferrets greater than 3 years of age. Characterization of lymph
nodes on ultrasound revealed hypoechogencity as the single most consistent
abnormality, as lymph node sizes were within reported ranges. Malignant changes seen
in cat and dogs, such as increased short to long axis length ratios rations, hyperechoic
perinodal fat, nodal heterogeneity, irregular nodal contour and shapes, were not
appreciated in ferret lymphoma cases (Suran). Splenic infiltration was noted but
correlation to splenomegaly should not be assumed, as extramedullary hematopoiesis
occurs in ferrets and this can confound assessment for the cause of splenomegaly.
Extranodal infiltration was then characterized in the liver, kidneys, lungs, and in
aggressive bone lesions.

Management

There are several modified chemotherapeutic protocols, which should be chosen based
on consultation with a knowledgeable oncologist. There are 3 common protocols that
have been adapted for use in ferrets with lymphoma, and BSA calculations have been
validated for the species, which is 9.94 x (body weight)^{2/3} (Jones 2015) with weight and
m^2 charts available. The Tufts protocol provides an intravenous-free 12 week
chemotherapy protocol that employs L-asparaginase, cyclophosphamide, cytabarne,
prednisolone, leukeran, and procarbazine, and methotrexate. The Gulf Coast
chemotherapy protocol utilizes a 52 week protocol that employs L'asparaginase,
prednisone, vincristine, and cyclophosphamide (Antinoff). Older protocols employ
vincritsine, asparaginase, prednisone, doxorubicin, cyclophosphamide, and
methotrexate. All physical protocols and dosages are available in the Ferret, Rabbit, and Rodents third edition chapter for Neoplasia in ferrets, (pp112-115). Copies available upon request. For localized cutaneous lymphoma, surgical excision of lesions may help improve quality of life, however recurrence is common without concurrent chemotherapy. Radiation therapy has been employed to reduce tumor size as rescue treatment for solitary mediastinal masses to relieve respiratory distress. While the tumors are very radio-sensitive, due to the ferret’s body conformation, limiting radiation exposure to other organs can be very challenging.

Adjunctive therapies include optimizing nutrition, screening for leukopenia (< 1,000) and neutropenia and providing systemic antibiotic therapy when indicated, and co-management of additional morbidities, which often include management of Helicobacter gastritis, and other neoplastic conditions (adrenocortical neoplasia, islet cell tumors). Avoid employing homeopathic therapies without consulting a knowledgeable specialists, as some treatments can and will cause harm. One such case has been proven in a clinical reports in dogs, receiving bloodroot (Sanguinaria canadensis) treatments, and the agent has been found to cause dermal necrosis (Childress).

Prognosis

Survival times strongly correlate with cell type and dissemination. Staging also heavily influences survival estimates, as disseminated T cell lymphoma may result in shorter estimates. In one study evaluating the phenotype, treatment and survival of 29 ferrets with lymphoma, Ammerbach et. al concluded that the mean survival of ferrets not immediately euthanized was 5.0 months (T-cell lymphoma) and 8.4 months (B-cell lymphoma). Ferrets treated with chemotherapy survived an average of 4.3 months (T-cell lymphoma, n = 9) or 8.8 months (B-cell lymphoma, n = 4). Ferrets in this study were diagnosed with peripheral T-cell lymphoma (n = 17), anaplastic large T-cell lymphoma (n = 5), anaplastic large B-cell lymphoma (n = 4), diffuse large B-cell lymphoma (n = 1), and Hodgkin-like lymphoma (n = 2).

Conclusion

Ferret lymphoma is one of the most common neoplasias recognized in practice. Clinical staging, grading, and phenotyping can help optimize treatment approach and qualify prognosis. Several chemotherapy protocols have been adapted for use in ferrets to help with ease of administration and improve compliance, improve quality of life, and reduce drug-induced morbidity.

References available upon request: llatney@vet.upenn.edu