Clinical presentation, treatment and outcome in 31 dogs with presumed primary colorectal lymphoma (2001-2013).

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The objective of this multicenter retrospective study was to describe clinical presentation, treatment, outcome, and determine prognostic factors for dogs with presumed primary colorectal lymphoma (PCRL). Thirty-one dogs were included. The predominant features of PCRL were high grade (n=18) and immunophenotype B (n=24). Most dogs were substage b (n=25) with higher prevalence of haematochezia (n=20). One dog had surgery only. Thirtydogs received chemotherapy; amongst them 13 had surgery or radiotherapy. Progression free survival (PFS) was 1318 days and disease-related median survival time (MST) 1845 days. Fourteen dogs were alive at the end of the study with a median follow-up time of 684 days (3- 4678 days). Younger dogs had longer PFS (p=0.031) and disease-related MST (p=0,01).Presence of haematochezia corresponded with longer PFS (p=0.02). Addition of local treatment to chemotherapy did not significantly improve the outcome (p=0.584). Canine PCRL has considerably longer PFS and MST than other forms of non-Hodgkin's lymphoma.

Keyword: canine, lymphoma, chemotherapy, colorectal

Abbreviations

AL: Alimentary Lymphoma

CI: Confidence Interval

CR: Complete Response

GI: Gastrointestinal

MST: Median Survival Time

PCRL: Primary Colorectal Lymphoma

PFS: Progression Free Survival

PR: Partial Response

Introduction

Canine gastrointestinal (GI) lymphoma, also named alimentary lymphoma (AL), is second in frequency to the multicentric form and accounts for 5-7% of all canine lymphomas.[1] In human medicine, AL is defined according to Dawson's criteria[2] (Figure 1), in which the lymphoma is considered alimentary only if the predominant lesion lies within the GI tract, with lymph node involvement confined to the lymph node chain draining that specific GI segment. In veterinary medicine, this definition historically has been extended and AL in general is described as the primary infiltration of neoplastic lymphocytes in the GI tract with or without additional extra-GI involvement confined to the abdominal cavity.[3] Most of the dogs affected by AL present late in the course of the disease with severe GI clinical signs and have rapid progression with a worse outcome than the multicentric form[4]: median survival time for canine AL ranges from 0.5 to 2.5 months,[4,5] versus 10 to 12 months for the multicentric form.[1] This difference in prognosis can be attributed to the negative prognostic factors carried by the most common presentation of canine GI lymphoma: T-cell immunophenotype, high grade and substage b.[4,5,6,7] However dogs with AL typically do worse than dogs with multicentric lymphoma carrying similar negative prognostic factors so it is unknown if there are additional inherent features of AL, such as specific morphologic subtype that could account for its poorer prognosis. Chemotherapy and supportive care are the standard of treatment for AL in dogs. A specific chemotherapy protocol (VELCAP-SC: vincristine, L-asparaginase, cyclophosphamide, doxorubicin, prednisolone - short, consolidated) for canine AL resulted in an overall median survival time of 77 days for the whole population and of 117 days for the chemotherapy responders.[4]

Primary large intestinal lymphoma can arise primarily from the colon, the rectum or both. In human medicine, it is referred to as primary colorectal lymphoma (PCRL) regardless of the segment of large intestine it is originating from. Canine PCRL is a rare presentation of AL, which has been sparsely described in the veterinary literature within case series reporting information about response and outcome for all GI locations.[3-6,8] When specific information for dogs with large intestinal lymphoma has been reported in those studies, a better outcome than other GI locations has been observed[3-5,8,9] with remission times up to 54 months (Table 1). A recent case series of 11 dogs with rectal lymphoma treated with surgery (7/11) and/or chemotherapy (8/11) described a mean survival time of 1697 days,

with a median not reached[10]. This data suggests that PCRL could have a better prognosis than AL in other locations. Such difference in prognosis could have implications in veterinary practice as some owners or veterinarians might be reluctant to treat dogs diagnosed with PCRL due to the currently described overall poor prognosis for AL. Questions that remain unanswered at the moment are: if studies with a higher number of patients would corroborate a distinct prognosis and a specific clinical presentation for canine PCRL; which prognostic factors are associated with this presentation and which therapeutic approach would have the best outcome. The purpose of the present multi-center retrospective study is to provide further information to answer those questions.

Material and methods

Case selection:

Dogs were presumed to have PCRL if they had absence of peripheral lymphadenopathy, a cytologic or histologic diagnosis of lymphoma had been made based on examination of a mass or diffuse intestinal wall infiltration of rectum, colon, or both and the main clinical signs at presentation were associated to large intestinal involvement. Dogs with concurrent abdominal lymphadenopathy, hepatomegaly and splenomegaly were eligible for inclusion. Exclusion criteria included presence of gross disease in other areas of the GI-tract evidenced by abdominal imaging or direct visualization at surgery.

The American College of Veterinary Internal Medicine Oncology Diplomate list serve was used to recruit cases for this retrospective study for which a diagnosis of PCRL was established. Medical records of dogs diagnosed with PCRL-between 2000 and 2013 at the Queen Mother Hospital for Animals, Royal Veterinary College, London, United Kingdom; The College of Veterinary Medicine, Washington State University, Pullman, Washington State, USA; East Bay Veterinary Specialists, Walnut Creek, California, USA; Animal Cancer Center, Colorado State University, Fort Collins, Colorado, USA; The Hope Center for advanced Veterinary Medicine, Vienna, Virginia, USA; The Veterinary Cancer Center, Norwalk, Connecticut, USA; School of Veterinary Medicine, Purdue

University, West Lafayette, Indiana, USA; Chavat Daat Veterinary Speciality Center, Beit Berl, Israel; Veterinary Medical Center, Michigan State University, East Lansing, Michigan, USA; Mississauga Oakville Veterinary Emergency Hospital and Referral Services, Oakville, Ontario, Canada; School of Veterinary Medicine, University of Madison-Wisconsin, Wisconsin, USA; Veterinary Specialty Center at Veterinary Emergency Service, Middleton, Wisconsin, USA; Southwest Veterinary Oncology, Gilbert, Arizona, USA; Alta Vista Animal Hospital , Ottawa, Ontario, Canada; Veterinary Medical Center, Ohio State University, Columbus, Ohio, USA; Animal Specialty and Emergency Center (ASEC), Los Angeles, California, USA; Vets Now Referrals, Blue Bell Hill, Kent, UK; were retrospectively reviewed.

Medical records review:

Data abstracted from the medical record included signalment, date and weight at diagnosis, presenting clinical signs, duration of the clinical signs, prior treatment(s), location of disease, method of diagnosis (cytology or histopathology), lymphoma grade and immunophenotype, results of clinical staging performed including physical examination findings, imaging technique used (thoracic radiographs, abdominal ultrasound, colonoscopy or CT scan) and bone marrow aspirate results, laboratory test results (including complete blood count, serum biochemical profile, urinalysis and faecal analysis when available), treatment modality (surgery, radiation therapy protocol, chemotherapy protocol(s)), response to therapy, date and cause of death or last follow-up visit. Necropsy results were recorded when available. Results of clinicopathological testing were classified as normal or abnormal by comparison with established reference ranges for the participating institution where the test was performed.

Histological reevaluation was performed for all cases for which samples were available. Lymphoma grading was performed with grading system employed at the discretion of the pathologist reviewing the slides for cases with histopathology and according to cell size for those with cytological diagnosis.[11-13] Immunohistochemistry or immunocytochemistry was performed using antibodies against CD3 and CD79 for all cases for which samples were available and in which

immunophenotyping had not been performed. Immunophenotyping was also performed with PCR for antigen receptor re-arrangement (PARR) in some cases.

Dogs were clinically staged at diagnosis according to the World Health Organization (WHO) criteria for canine lymphoma.[1] Dogs were further classified as substage a (having no clinical signs) or substage b (having systemic signs or clinical signs associated to the gastrointestinal presentation of lymphoma with potential impact on systemic status, as previously described for assessment of substage in canine AL). Clinical signs included in the b substage category were haematochezia, tenesmus, diarrhoea, vomiting, lethargy, or decreased appetite. Haematochezia, tenesmus, vomiting, and diarrhoea were considered local clinical signs; lethargy and decreased appetite were considered systemic signs. Information regarding exact anatomic location and extent of the disease was gathered from the physical examination and staging test results.

Therapeutic modality was recorded for each patient. When surgery was performed, information regarding the type and extent of surgery (i.e. excisional or incisional biopsy, date of procedure, and regional lymph node(s) removal) was recorded. Only dogs that had an excisional surgery were considered to have had surgery as a treatment. Surgical technique was recorded when available. For patients that were treated with radiation therapy, type and protocol (dose, number and frequency of fractions) were recorded. For chemotherapeutic treatments, information regarding protocols (induction and rescue) including drug, dose, administration frequency and protocol duration, was recorded. For descriptive purposes, the first chemotherapy protocol used was categorised as: CHOP type protocol, doxorubicin single agent, COP type protocol, lomustine-based protocol, prednisolone and chlorambucil or prednisolone alone. CHOP protocols could be of different length (19 or 25 weeks) and sometimes included L-asparaginase, or epirubicin instead of doxorubicin; the COP type protocols sometimes included a dose of cytarabine (COAP protocol).

Response to treatment was classified according to remission status recorded by the veterinarian providing the data using the following categories: complete response (CR), partial response (PR), stable disease, and progressive disease. Response to treatment was mainly determined based on results of clinical examination and assessment of progression of clinical signs. When available, follow-up abdominal ultrasound examinations were used to assess for changes in internal organs. Complete

response was defined as resolution of all clinical signs and disappearance of all clinical evidence of disease on the basis of physical examination (via rectal and abdominal palpation) or follow-up abdominal ultrasound examination. Partial response was defined as (≥) 50 and (<) 100% reduction in size of measurable disease on the basis of physical examination (via rectal and abdominal palpation) or follow-up abdominal ultrasound examination associated or not with improvement of the clinical signs. No response was defined as no changes or deterioration in clinical signs, and less than 50% reduction, increase in the size of measurable disease or appearance of new lesions on the basis of physical examination (via rectal and abdominal palpation) or follow-up abdominal ultrasound examination.[14] When surgery was performed prior to chemotherapy, only dogs with residual macroscopic disease were included in the assessment of response to chemotherapy.

Statistical analysis:

Categorical data are presented either as percentages or ratios. Continuous data are presented as median (range). Survival time was defined as the time from diagnosis until natural death or euthanasia. Deaths due to disease progression were considered events for the disease-related median survival time. Dogs that were lost to follow-up, still alive at the end of the study period or died from causes unrelated to the lymphoma were censored. In addition, overall survival time was also calculated. Due to the small number of patients that died from lymphoma, this variable included all deaths as events (related to lymphoma or unrelated) with dogs lost to follow-up or still alive at the end of the study period being censored. Progression free survival was defined as the time from initiation of treatment to event. For this variable the date of relapse was considered as the endpoint for dogs when this occurred. Dogs that had not relapsed during the study period were censored at the last date they were contacted or evaluated by the vet or the date of death free of disease.

After statistical description of the patient population, survival analysis using Kaplan-Meier product limit method was conducted to estimate disease-related median survival time (MST), median overall survival time, and progression free survival (PFS), for the whole population of PCRL patients. Disease-related MST and PFS of dogs distributed in groups on the basis of various potential risk

factors (univariate analysis) was also calculated. Exploratory statistical analysis for each categorical risk factors (age, location of the disease, involvement of organs other than the large intestine, substage, number of clinical signs present, type of clinical signs (local vs systemic), grade, immunophenotype, treatment type, chemotherapy protocol) used the logrank test to compare estimated PFS and disease-related MST between categories. Multivariate analysis was not performed due to the small number of animals in each group and high censorship of dogs in the study. A value of $p \leq 0.05$ was considered significant. All calculations were performed with the aid of a standard statistical software[a].

Results

Signalment: Thirty-one dogs met the inclusion criteria for this study and consisted of 17 spayed females and 14 males (12 neutered and 2 intact). The median age at diagnosis was 5 years (range 1.5-13.5 years old). Median weight was 23.8 kg (range 5.9-52.1 kg). The most common breeds were cross breeds (7 dogs; 22.6%), and Labrador retrievers (4 dogs; 12.9%).

Clinical signs at diagnosis: Presenting clinical signs were known for 29 of 31 dogs. Twenty-five (80.64%) dogs had clinical signs at presentation associated with the disease and were considered subtage b. Haematochezia was the most common presenting clinical sign (n = 20; 64.5%) with 13 of these dogs presenting additional clinical signs. Other reported clinical signs included: tenesmus in 35.6 % (n=11), diarrhea in 25.8 % (n=8), narrow diameter of faeces in 12.9% (n=4), vomiting in 3.4% (n=1), lethargy in 3.4% (n=1). Eighteen dogs (58%) had simultaneously 2 to 4 different clinical signs and 7 dogs (22.5%) had one clinical sign at presentation. Presence of a rectal mass was described on physical examination in 35.6 % (n=11) and rectal prolapse in 9.68% (n=3). The median duration of clinical signs was 21 days (range 1-120 days) for the 28 dogs for which this information was available.

Staging and location of the disease: As per inclusion criteria, none of the dogs had peripheral lymphadenopathy identified on physical examination. Imaging was performed in 29 patients: 2 dogs had imaging of thorax and abdomen as well as bone marrow evaluation, 19 had imaging of the thorax

and abdomen, 7 dogs had only abdominal imaging and 1 had only thoracic imaging. Abdominal imaging consisted of ultrasonography in 24 dogs, radiographs in 2 and computed tomography in 2. Thoracic imaging was performed in 2 dogs with CT scan and in 20 with radiographs. Dogs that did not have abdominal imaging had direct evaluation of the GI tract via laparotomy or endoscopy.

A solitary mass was found in 23 patients (17 rectal masses of which 4 dogs also had regional lymph node involvement, 6 colonic masses of which 5 dogs also had regional lymph node involvement). Multiple masses were identified in 5 patients, with 1 dog having multiple masses in the rectum, 1 dog with multiple masses in the colon, and 3 dogs having masses occurring in both areas. Three dogs were determined to have lymphoma diffusely throughout the colorectal region. Regional lymphadenopathy was present in 12 dogs with lymphoma confirmed by lymph node cytology for 8 dogs. Splenic aspirates were performed in 2 dogs and liver aspirates in 4 dogs. Liver involvement was confirmed in 1 dog based on cytology.

Immunophenotype and grade: Three cases were diagnosed via cytology and 28 by histopathology. Histological or cytological assessment of lymphoma grade was reported for 29 cases (94%) and 2 cases were reported to be low grade lymphoma, 9 were intermediate grade lymphoma, and 18 high grade lymphoma (Table 2). Slides of 22 cases were obtained for review by a pathologist (7 different pathologists reviewed all the slides with 2 of them reviewing a total of 16 slides- KS and BP), in those confirmation of diagnosis and grade was performed. None of the cases which had a grade previously done and for which histopathology slides were reviewed had a change in their grade.

Immunophenotype was available for 26 patients (84%). It was performed via immunohistochemistry for 23 dogs and this took place at the time of pathology review for 12 cases. Immunophenotype was determined by immunocytochemistry for 2 dogs and PARR for 1 dog. There were 24 B-cell lymphomas, 1 T-cell lymphoma and 1 non-B non-T cell lymphoma. (Table 2).

Treatment and response to treatment: All dogs received therapy, and all but one (that had only surgery) received chemotherapy as part of the treatment plan. Seventeen patients (55.5%) received chemotherapy as a sole treatment and thirteen (42%) received chemotherapy in addition to local

treatment. Nine dogs (29%) had surgery prior to adjuvant chemotherapy. Four dogs (13%) had chemotherapy combined with radiation therapy. These cases received radiation therapy with photons; protocols comprised hypofractionated regimens for 3 dogs (2 dogs received 2 fractions of 6 Gy and one dog received one fraction of 6 Gy) and the fourth dog was treated with a hyperfractionated protocol (19 fractions of 1.5 Gy). One dog (2.5%) had surgery alone.

Out of the ten patients that had surgery, nine dogs (29%) presented with a solitary mass. The remaining dog had a solitary mass removed from the rectum and was found to have diffuse disease in the colon at later staging. Two dogs also had lymph node involvement and one of them had the lymph nodes biopsied at the same surgery. Eight dogs (25.8%) had surgery performed by the referring vet prior to referral and there was no evidence of previous diagnosis based on cytology or biopsy for the two remaining dogs so it is likely that surgery in the 10 patients was performed as a mean to obtain a diagnosis as well as therapeutic procedure. Due to the retrospective nature of the study, the primary intent of performing surgery could not be ascertained. Type of surgery was recorded for 2 patients: one had mucosal resection and one had end-to-end anastomosis. The latter procedure was performed because the dog was initially referred for a rectal polyp previously diagnosed on endoscopic biopsies performed by the referring vet and lymphoma was diagnosed on the surgical histopathology. Eight patients out of the 28 diagnosed via histopathology (25.8%) had endoscopic biopsies.

The distribution of induction chemotherapy protocols used was as follows: 18 (58.1%) patients received a CHOP type protocol, 5 dogs (16%) had a COP type protocol, 3 (10%) had a lomustine-based protocol (lomustine / vincristine / cyclophosphamide; lomustine / prednisolone or lomustine / prednisolone/ L-asparaginase respectively), 1 dog (2.5%) had doxorubicin single agent and prednisolone, 1 dog (2.5%) had chlorambucil and prednisolone , and 2 dogs (5%) received only prednisolone. The response rate to chemotherapy for the 22 dogs that had gross disease at the start of chemotherapy (only one of the dogs that had surgery had gross residual disease at the start of chemotherapy) was available in the records of 19 dogs (86%). The overall response rate was 100% with 18 dogs (95%) having a complete response and 1 dog (5%) a partial response.

The estimated PFS was 1318 days, since 71% of dogs were censored the confidence interval (CI) is not available. Nine patients had a relapse during the follow up period and reinduction with a chemotherapy protocol was attempted in 8 of them (six dogs received one additional protocol, one dog received 2 and one received 5 additional protocols). Chemotherapy protocols used for reinduction comprised: lomustine/prednisolone with or without L-asparaginase, CHOP, COP, vincristine/chlorambucil, doxorubicin single agent or vincristine single agent. Two dogs were euthanized the same day that the chemotherapy was resumed. Response was available for 5 out of the 6 remaining dogs and all had a complete response. The median remission duration for these 5 dogs, after the first reinduction protocol was initiated, was 411 days (range 298-938).

Outcome: At the end of the study period, 14 dogs were still alive, 5 dogs were lost to follow up, 7 dogs died of lymphoma, and 5 dogs died of unrelated causes (1 intramuscular hemangiosarcoma, 1 suspected osteosarcoma, 1 chronic kidney disease, 2 unknown cause). Only one dog underwent necropsy and disseminated lymphoma was found to be the cause of his death. The median follow-up time was 684 days (range 3-4678 days). The estimated disease-related MST where only dogs that died from lymphoma were considered as events was 1845 days (Figure 2). Since 77.5% of dogs were censored, the CI was not available. The estimated overall MST, where dogs that died from any causes were considered as events, was 1548 days (95% CI: 886- 2210). MST calculated only for those 7 patients that died of lymphoma was 643 days (range 399-1845 days) and for the 5 patients dying of unrelated causes was 789 days (range 465-4678 days). The 1, 2, and 4 year overall survival rates for all dogs in this study were 100, 50, and 25 %, respectively.

Prognostic factors:

There was no statistically significant association between gender, number of presenting clinical signs (one versus multiple), type of clinical signs (systemic versus local), location of the disease (solitary vs multiple/diffuse), involvement of organs other than intestines, immunophenotype (B vs others), grade (high vs others), treatment (chemotherapy alone versus chemotherapy plus a local treatment) (Figure 3), chemotherapy treatment (CHOP/doxorubicin-based protocol versus others or CHOP vs others) and

either PFS or disease-related MST. The disease-related MST for dogs receiving chemotherapy alone was 1845 days (95 % CI: 855-2835) versus a median that could not be reached for dogs receiving chemotherapy plus a local treatment and the difference between these medians was not statistically significant (p-value 0.584) (Figure 3). When substage was analysed it was not a statistically significant risk factor for disease-related MST (p-value = 0.345) but substage b was found to have a significantly positive impact on PFS (p-value = 0.001), with a PFS that was not reached for dogs categorized as substage b (95% CI: 0-2331.5) versus a PFS of 271 days for dogs without clinical signs at diagnosis. The presence of haematochezia at diagnosis was not a statistically significant risk factor for disease-related MST (p-value = 0.147) but was found to have a significantly positive impact on PFS (p-value = 0.02), with a PFS of 1141 days for dogs without haematochezia (95% CI: 0-2331.5) versus a PFS that was not reached for dogs with haematochezia at presentation. Dogs younger than 7 years had longer PFS than dogs older than 7 years (836 days for older dogs, 95% CI: 537-1135 versus a PFS not reached for younger dogs, p-value = 0.031 and 0.01 respectively) but a similar disease-related MST was present in both groups.

Discussion

The primary goal of the current study was descriptive, as PCRL has not previously been described in a large case series in the veterinary literature and just few sparse cases of colorectal lymphoma have been reported (Table 1). The current study showed that PCRL occurs primarily in middle-aged dogs with no sex or breed predisposition. Results of this study suggested that PCRL is typically high grade and B cell immunophenotype with most dogs presenting as substage b. The majority of dogs (23/31; 74.2%) presented with a solitary mass and 13/31 (43.7%) had evidence of involvement of abdominal extra GI organs (liver or regional lymph nodes). The disease features of dogs with PCRL described in this study are consistent with cases previously reported.[3-5,8,10,15]

Results of the present study suggest that dogs with PCRL treated with chemotherapy, with or without additional local therapy, had a very good prognosis as demonstrated by a high response rate, long

PFS, and long MST. This is in agreement with previous published results [3-5,8,10,15], and in contrast to the reported low response rate (56%) [4] and short survival time (13-77 days) for dogs with diffuse GI tract lymphoma of other locations.[4,5] The CI could not be calculated for disease-related MST, despite a median follow-up time of almost 2 years, as 14 (45%) of the dogs in this study were still alive at the time of writing. Therefore it is possible that the PFS and survival time for dogs with PCRL might be even longer than has been estimated in our study. For a more accurate determination of disease-related MST, the population in this study would require follow up beyond 2 years. The possibility of additional deaths unrelated to lymphoma for dogs currently alive and in complete remission besides the 5 already recorded, could continue adding censored dogs to the statistical analysis which will make it difficult to reach a defined value for PFS and disease-related MST. We considered that the improved prognosis of dogs with PCRL found in this study, particularly in comparison to previously published outcomes of dogs with AL lymphoma, warranted communication to the veterinary community despite availability only of estimated statistical parameters via the Kaplan Meier method.

A subsequent goal of our study was to evaluate outcome with different treatment modalities for dogs with PCRL. No statistical difference was found between PFS or survival of dogs receiving chemotherapy only versus chemotherapy plus local treatment with surgery or radiation. Patients that received chemotherapy only had a PFS of 1318 days and a disease-related MST of 1845 days, and the PFS and disease-related MST were not reached for patients that had chemotherapy combined with local treatment, however, this difference in outcome was not statistically significant. This finding shows that chemotherapy alone was effective to treat a localized presentation of lymphoma and surgery or radiation therapy, which carry some morbidity, might delay start of medical anticancer treatment, and increase total cost of therapy, are not necessarily required to achieve a good outcome.

In this study surgery was performed mainly in cases with presumed solitary large intestine masses primarily to achieve a diagnosis and we lack information documenting if alternative diagnostic procedures were previously attempted unrewardingly or why surgery was elected by referring vets as a diagnostic tool above other diagnostic modalities. It is possible that the differential diagnosis of PCRL and its implications in the therapeutic approach were not considered due to its rarity in

comparison to other types of colorectal neoplasia for which surgery is routinely recommended. The findings in this study support consideration of PCRL as a differential for solitary colorectal masses and when possible the use of low invasive diagnostic techniques such as cytology or incisional biopsy prior to treatment planning. Due to the retrospective nature of the study, treatment groups were not randomised and there is a possibility that surgery had been selected more often for dogs with more advanced diseases and that multimodality treatment would be more beneficial than a single treatment for advanced disease. On the contrary surgery may also have been elected more often for dogs with isolated disease, and it might have made a difference in the outcome of PCRL patients, as this has been described for solitary lesions of lymphoma in other locations.[16-18]

In this study there was no difference in PFS or survival time based on the type of chemotherapy protocol used to treat dogs with PCRL. We have to be very cautious in the interpretation of our data considering that, due to the small number of dogs in the present study, only two chemotherapy groups could be defined (doxorubicin-based protocol vs others). Still, it is important to consider that these results describe that less intense chemotherapy protocols, often chosen when facing client economic or time constraints or when clients seek therapies with potentially lower prevalence of adverse effects, seem to achieve similar PFS and survival times than with CHOP type protocols. This finding correlates with a previous studies where an overall survival time of approximately 4.5 years was reached in 9 dogs with rectal lymphoma receiving either a low dose COP protocol or a truncated (6 weeks) CHOP protocol[10].

Age greater than 7 years was associated with a significantly decreased prognosis as compared to dogs younger than 7 years. Since most dogs had long PFS, it is possible that older patients were more likely to succumb to concurrent or subsequent morbidities than younger dogs. Decreased owner motivation to re-start treatment at relapse in older dogs is also a possible reason that these dogs had a shorter disease-related MST as compared to younger dogs, but this would not explain the shorter PFS experienced by dogs older than 7 years.

Canine lymphoma patients with systemic signs or clinical signs associated to their presentation with a systemic impact (substage b) are known to have a worse prognosis.[1,19-21] Dogs with AL are often substage b at diagnosis mainly due to clinical signs associated to local disease and it is possible that they often are less responsive to therapy due to clinical signs being surrogate markers of more advanced disease.[5,14] GI signs, particularly upper GI signs such as anorexia and vomiting, are often perceived as poor quality of life by owners, leading to an early decision of euthanasia during the course of treatment. In the present study, although most dogs presented with clinical signs associated to PCRL, their symptoms did not appear as severe in nature as GI signs of dogs with diffuse small intestinal or gastric lymphoma. We decided to consider haematochezia, tenesmus, and diarrhoea as markers of systemic illness due to the possible associated systemic discomfort and pain and potential impact in quality of life. Being this a retrospective study with limited recorded information about severity of clinical signs and impact on the dogs' general status, grading of GI signs at presentation according to the VCOG criteria[22] was not possible and precluded a comparison with previous studies about canine AL. Interestingly in this study there was no difference in outcome for dogs with systemic versus local signs and substage did not have an impact in survival time. Although PFS of dogs without clinical signs was shorter than for dogs with substage b (271 days versus not reached), these results could have been biased due to the small number of dogs in the substage a group or potential missing data regarding patient's clinical signs. More information is needed to ascertain how best to apply the current canine lymphoma staging system to extranodal forms like PCRL and to identify its value in determining prognosis.

The presence of haematochezia at diagnosis was found to have a positive impact on the PFS compared to other signs. For the 20 dogs presenting with haematochezia, the PFS was not reached, compared with 1141 days for 9 dogs without haematochezia but with other clinical signs. It is not clear why haematochezia would have a positive impact on the prognosis. Perhaps this was more alarming for owners than diarrhoea and its observation might have led to earlier diagnosis and treatment. On the other hand, we can also assume that other signs reported (diarrhoea, rectal prolapse, tenesmus) might be associated with more advanced disease and decreased outcome, however 13/20

cases with haematochezia also had additional clinical signs. This result could also be secondary to a statistical bias; a larger population would help to determine if the presence or absence of haematochezia truly has an impact on prognosis.

In previous studies on canine AL, the immunophenotype was most commonly of T-cell origin.[5,6,8,23-25] We report here that colorectal lymphoma is most commonly of B-cell origin, raising a clear distinction from other GI locations. The B-cell phenotype in canine high-grade lymphoma generally is associated with a better response to conventional chemotherapy protocols, compared with the T immunophenotype.[19,26] In the current study, 77% of the dogs were of immunophenotype B, there were only 2 cases of known non-B immunophenotype with outcomes comparable to the ones with immunophenotype B (survival times 491 and 1273 days) and 5 cases with unknown immunophenotype (Table 2). We did not find any impact of immunophenotype on outcome but the low number of dogs with non-B phenotype included in the study clearly precludes knowing the real impact of this factor in PCRL.

The most common grade for lymphoma found in this study was high (58% of the dogs) and was not a significant prognostic factor in our population. The grading was performed based on the National Cancer Institute working formulation with separation of the patients into 3 categories: low, intermediate, and high grade.[11-13] Recent studies advise for classification of canine lymphomas according to the WHO classification [7,28] in order to standardise future study populations as this may have a prognostic significance.[27] This would be extremely interesting for canine PCRL in order to further characterize and describe this anatomical presentation. However, in this retrospective study, the limited availability of initial tissue samples and absence of immunophenotype for some dogs has precluded this classification. Other studies that classified their patients according to the working formulation into low, intermediate, and high grade[13] found that grade had prognostic significance.[12] In our study, there were too few dogs in the low or intermediate grade category and perhaps this could have an impact on the statistical significance. Interestingly, despite most dogs having high grade B cell lymphoma, their disease-related MST was much longer (1845 days) than that of dogs affected by the multicentric form of same grade and immunopenotype (MST reported in literature of 162-308

417 days[12]), reinforcing the fact that anatomic site seems to be a better predictor of outcome than
418 previously published prognostic factors.

419 Information available for staging of dogs included in this study was limited due to its retrospective
420 nature and staging performed was quite heterogeneous due to the high number of participating
421 institutions. This was unfortunately unavoidable when trying to compile a large case series of PCRL
422 due to its low prevalence. Staging was not complete for all dogs and different imaging modalities
423 were used. In addition, even imaging techniques used routinely for staging in veterinary oncology
424 such as abdominal ultrasound or computed tomography have limitations for detection of lymphoma.
425 Also aspirates of liver and spleen were not routinely performed despite knowledge that neoplastic
426 infiltration is possible even with a normal imaging appearance[29]. In this study, twelve dogs had
427 evidence of regional lymphadenopathy and one hepatic involvement without impact on PFS or
428 survival time. It is very possible that a higher number of dogs had a higher stage of disease than
429 recorded leading to erroneous staging classification of cases (stage migration)[30] and this could explain
430 the lack of statistical difference. On the other hand, given the overall long PFS and survival times for
431 all patients, it could also be thought that possibly under-staged dogs had a good outcome. Based on
432 this study's data it seems that presence of complete staging would not have provided any further
433 prognostic information for dogs with PCRL. However, as there is not established standard treatment
434 for PCRL, it would be advisable that complete staging would be performed to outweigh potential
435 advantages versus morbidity/cost if considering local therapy prior to chemotherapy or as solely
436 therapy in PCRL.

437 Human primary AL are classified as confined to the GI tract, with no other evidence of systemic
438 involvement (see figure 1). They are most often of B cell origin[8], which is comparable to our study
439 findings, but opposite to the immunophenotype of canine AL[6]. PCRL is a rare disease in human
440 medicine[31] with controversy about what the standard of care should be for its treatment.[31] Long
441 survivals have been described with 83% of the patients alive and free of disease at 10 years after a
442 combination of surgery and chemotherapy.[32] but shorter prognosis with a 5 year survival rate of 47%
443 has also been reported.[33] In people, the two most frequent histologic subtypes of PCRL are mucosa-

associated lymphoid tissue (MALT) lymphoma and diffuse large B cell lymphoma with both PCRL types carrying very different prognosis. The MALT form, of low grade and B-cell origin, can be cured after surgical resection and/or a short course of chemotherapy. The prognosis is more guarded for the diffuse large B cell lymphoma which carries a high relapse rate (33-75%).[34] Interestingly, although most cases of canine PRCL in our population were high grade and B-cell origin the biologic behaviour seems to differ from the high grade form of human PRCL.

The limitations of this study are inherent to it retrospective design and include small sample size, variable staging and treatment regimens, and lack of standardized follow-up. The small population due to the rarity of the disease limited the statistical power of our analyses and prognostic factors associated with PFS and disease-related MST may not have been identified due to the variations in staging and treatment protocols. Of the factors analysed, few were found to be prognostic indicators in exploratory univariate analysis. Unfortunately, multivariate analysis could not be carried out as too many dogs were censored mostly due to long survival, loss of follow-up, or death from unrelated cause. Evaluation of a larger sample size, prospectively, over a longer period of time including standardized comprehensive staging and standardized therapy would be ideal and may permit determination of additional characteristics and identify risk factors for PCRL. This would be challenging, requiring even further collaboration than the one required for this study (with 18 institutions collaborating in 4 countries) due to the low prevalence of this presentation of canine lymphoma. It needs to be reinforced that larger number of dogs and longer follow-up may still not reach statistical significance if most patients do not demonstrate events linked to their lymphoma (relapse or death) as they would remain censored from the whole population should they remain in remission or die from other causes.

In conclusion, results of the present study describe canine PCRL as a rare disease often of high grade and B-cell immunophenotype that when treated with chemotherapy results in prolonged PFS and survival times regardless of chemotherapy protocol type or addition of local treatment modalities. Results of this study suggest that PCRL has a greatly improved outcome compared to previously published outcomes of dogs with AL. Therefore, attempts to differentiate dogs with PCRL from dogs

with diffuse GI lymphoma should be pursued in order to better guide owners regarding treatment and associated prognosis. Veterinarian awareness of this specific presentation of canine lymphoma will likely have an important impact on the treatment decision process for owners of dogs with PCRL.

Footnotes

a. (SPSS version 19.0 for Windows, SPSS Inc, Chicago, Ill).

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Table 1: Cases of rectal lymphoma in the veterinary literature (LN: lymph node, NA: not available)

Cases (n)	Location	Diagnostic method	Immuno-phenotype	Treatment type	Survival	Ref.
1	Large intestine	NA	NA	Chemotherapy: COAP protocol	>54 months	3
7	Rectum	histology	NA	Surgery	5 weeks, 1 dog had no relapse after 8 months, 1 relapsed but was still alive at 18 months	9
6	Large intestine	5 histology 1 cytology	NA	4 chemotherapy only (type NA) 2 surgery + chemotherapy (NA)	61 days (0-2520 days)	5
1	Colon	histology	T	VELCAP-SC protocol	NA	4
1	Rectum	histology	NA	COP protocol	> 356 days	8
11	Rectum	histology	10 B	6 surgery + chemotherapy (CHOP/COP) 1 surgery alone 3 chemotherapy (CHOP/COP or prednisolone) 1 no treatment	1697 days	10

609 Table 2: Location, extension, histologic characteristics, and treatment data of dogs with colorectal
610 lymphoma.

Data	Number of cases
Aspect	23 solitary masses 5 multiple masses 3 diffuse disease
Location	18 rectal disease 7 colonic disease 6 colorectal disease
Staging	12 lymph node involvement 1 liver involvement
Substage	29 substage b 2 unknown
Immunophenotype	24 B 1 T 1 non B non T 5 non available
Grade	18 high 9 intermediate 2 low 2 non available
Treatment	1 surgery 17 chemotherapy 9 chemotherapy + surgery 4 chemotherapy + radiotherapy
Chemotherapy protocol	18 CHOP 5 COP 1 doxorubicin 3 lomustine 1 chlorambucil + prednisolone 2 prednisolone

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Figure 1: Criteria of inclusion for human primary gastrointestinal lymphoma

Dawson's criteria:

- No generalized, superficial, or mediastinal lymphadenopathy
- No leukemic or lymphomatous abnormalities in the peripheral blood
- Lesion confined to the bowel, with only regional lymphadenopathy at the time of laparotomy
- No involvement of the spleen or the liver at the time of diagnosis

Figure 2: Kaplan Meier of the estimated survival time with the event being dogs that died from lymphoma. The estimated MST was 1845 days (confidence interval not available).

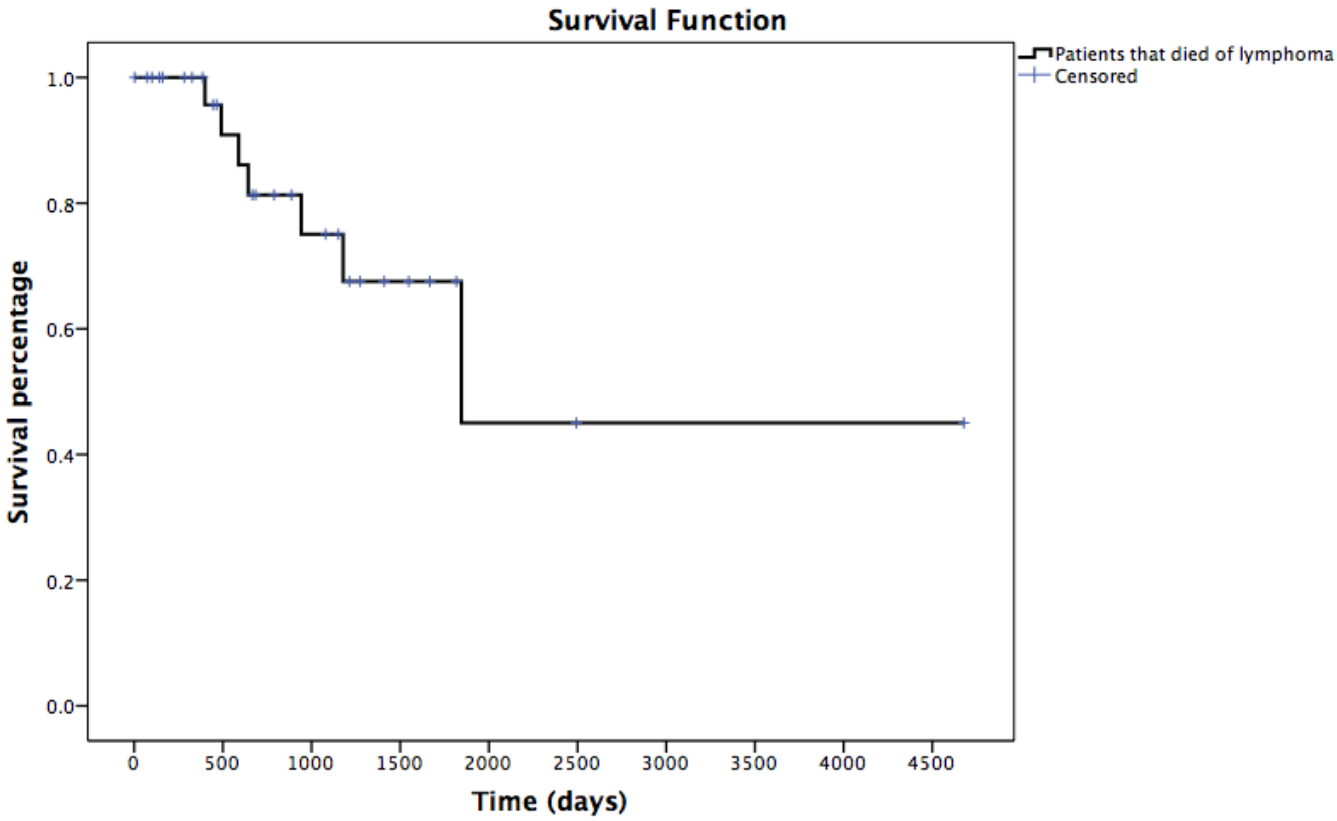

Figure 3: Kaplan Meier of the estimated survival time for dogs receiving chemotherapy versus dogs receiving chemotherapy plus a local treatment. The addition of a local treatment to chemotherapy did not impact the overall prognosis (p-value = 0.564).