Title

Retrospective evaluation of clinical outcome after chemotherapy for lymphoma in 15 equids (1991-2017)

Journal

Journal of Veterinary Internal Medicine, 33(2)

ISSN

0891-6640

Authors

Luethy, Daniela

Frimberger, Angela E

Bedenice, Daniela

et al.

Publication Date

2019-03-01

DOI

10.1111/jvim.15411

Received: 11 August 2018 | Accepted: 12 December 2018

DOI: 10.1111/jvim.15411

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

STANDARD ARTICLE

Retrospective evaluation of clinical outcome after chemotherapy for lymphoma in 15 equids (1991-2017)

Daniela Luethy[1] | Angela E. Frimberger[2] | Daniela Bedenice[3] | Barbara S. Byrne[4] | Erin S. Groover[5] | Rachel B. Gardner[6] | Trisha Lewis[7] | Valerie S. MacDonald[8] | Lauren Proctor-Brown[9] | Joy E. Tomlinson[9] | Kenneth M. Rassnick[10] | Amy L. Johnson[1]

[1]Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, Pennsylvania

[2]Veterinary Oncology Consultants, Wauchope, New South Wales, Australia

[3]Department of Clinical Sciences, Cummings School of Veterinary Medicine at Tufts University, North Grafton, Massachusetts

[4]Department of Pathology, Microbiology and Immunology, University of California Davis School of Veterinary Medicine, Davis, California

[5]Department of Clinical Sciences, Auburn University College of Veterinary Medicine, Auburn, Alabama

[6]B.W. Furlong & Associates, Oldwick, New Jersey

[7]Lewis Veterinary Services, Maple Park, Illinois

[8]University of Saskatchewan Western College of Veterinary Medicine, Saskatoon, Saskatchewan, Canada

[9]Baker Institute for Animal Health, Cornell University College of Veterinary Medicine, Ithaca, New York

[10]Veterinary Medical Center of New York, East Syracuse, New York

Correspondence
Daniela Luethy, Department of Clinical Studies-New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, 382 West Street Road, Kennett Square, PA 19348.
Email: dluethy@vet.upenn.edu

Background: Prognosis associated with lymphoma in horses is poorly characterized, and treatment is often palliative. Long-term outcome after chemotherapy for horses with lymphoma is not well documented.

Objective: To report long-term outcome of horses with lymphoma treated with chemotherapy.

Animals: Fifteen equids.

Methods: Retrospective case series. Medical record search and call for cases on the ACVIM listserv for horses treated with chemotherapy for lymphoma.

Results: Fifteen cases with adequate data were identified. Complete remission was achieved in 5 horses (33.3%), partial response was achieved in 9 equids (60%), and stable disease was achieved in 1 horse. Overall response rate was 93.3% (14/15). Overall median survival time was 8 months (range, 1-46 months). Nine horses experienced a total of 14 adverse effects attributable to chemotherapy. Adverse effects were graded according to the Veterinary Cooperative Oncology Group common terminology criteria for adverse events grading system (grade 1 alopecia, n = 2; grade 1 neutropenia, n = 2; grade 1 lymphopenia, n = 3; grade 1 lethargy, n = 1; grade 2 neurotoxicity, n = 1; grade 2 colic, n = 1; grade 1 hypersensitivity, n = 1; grade 2 hypersensitivity, n = 2; grade 5 hypersensitivity, n = 1). Higher grade adverse effects most commonly were associated with doxorubicin administration (n = 4), including 1 horse that died 18 hours post-administration.

Conclusions and Clinical Importance: Chemotherapy can be used successfully for treatment of horses with lymphoma. Adverse effects, most commonly mild, occurred in approximately two-thirds of treated horses.

KEYWORDS

chemotherapy, doxorubicin, horse, neoplasia

Abbreviations: CR, complete remission; EHV, equine herpes virus; PR, partial response; SD, stable disease; TCRLBCL, T-cell rich large B-cell lymphoma.

1 | INTRODUCTION

Lymphoma, the most common malignant neoplasia seen in horses, represents 1%-14% of tumors in horses.[1] Lymphoma in horses can be classified by anatomic distribution into alimentary, cutaneous, multicentric, or mediastinal forms. Clinical presentation varies depending on the sites affected. In a previous large retrospective report of lymphoma in horses, which classified cases based on histomorphology and immunophenotyping, the multicentric form was most common (41%), followed by cutaneous (19%) and alimentary (11%) forms.[2] That study also found T-cell rich large B-cell lymphoma (TCRLBCL) to be the most common histologic subtype seen in horses with lymphoma, whereas peripheral T-cell lymphoma and diffuse large B-cell lymphoma also were seen frequently.[2]

Prognosis for horses with lymphoma is variable and not well characterized. Treatment is often palliative and may include surgical excision, corticosteroids, chemotherapy, or a combination of treatments. Description of the use of chemotherapy for treatment of horses with lymphoma has been limited to case reports,[3,4] and no study has evaluated a large number of horses treated by chemotherapy to determine the efficacy of treatment and long-term outcome in horses with lymphoma. Our primary objective was to report long-term outcome of equids with lymphoma treated using chemotherapeutic protocols.

2 | MATERIALS AND METHODS

Cases were identified by search of electronic and hard copy medical records from 2006 to 2017 at the New Bolton Center Large Animal Hospital at the University of Pennsylvania and by an email call for cases to the ACVIM Large Animal Internal Medicine and Oncology Diplomate listservs (no dates specified). Case inclusion criteria consisted of (1) equid patients with (2) histologically confirmed lymphoma that were (3) treated using chemotherapeutic protocols. Horses were excluded if the diagnosis was presumptive and not confirmed by histology, if chemotherapeutic treatment was not attempted or if no follow-up information was available. Information recorded for each patient included signalment, history, presenting complaint, physical examination findings, clinicopathologic data, stage, immunophenotype and anatomic form of lymphoma, treatment (chemotherapy, corticosteroids, surgical excision), adverse events, long-term outcome, and necropsy results (where applicable). Lymphoma was classified based on anatomic distribution, and multicentric lymphoma was defined as involving at least 2 organs, not including regional lymph nodes, as previously described.[2] Response to chemotherapy was described using previously reported criteria.[5] Briefly, complete remission (CR) was defined as disappearance of all evidence of disease and clinical signs. Partial response (PR) was defined as >30% decrease in the size of baseline pathologic lesions. Progressive disease was defined as >20% increase in the size of lesions. Stable disease (SD) was defined as <30% decrease or <20% increase in the size of lesions. Time to reassessment and method of reassessment varied and were not standardized. Overall response rate was defined as (CR + PR)/total cases.

2.1 | Statistical analysis

Descriptive statistics were used to report clinicopathologic findings. Numerical values are reported as medians and ranges unless otherwise specified. Survival analysis was performed using the Kaplan-Meier method.

3 | RESULTS

3.1 | Cases

Fifteen equids met the inclusion criteria. Median age was 11 years (range, 4-25 years). There were 6 Warmbloods, 3 Quarter Horses, 2 Standardbreds, 1 Arabian, 1 Thoroughbred, 1 Tennessee Walking Horse, and 1 Donkey. These included 5 mares, 9 geldings, and 1 stallion. Two mares, both Standardbreds, were in foal (120 and 150 days pregnant at time of diagnosis). The case description of 1 of these mares had been reported previously in a conference abstract.[6]

3.2 | Classification

Anatomic distribution of lymphoma was classified as multicentric (n = 9), cutaneous (n = 3), and alimentary (n = 3). Ten horses had immunohistochemistry performed on biopsy specimens to determine immunophenotype: 6 cases were histologically classified as TCRLBCL, 2 were classified as large B-cell lymphomas, and 2 were classified as T-cell lymphoma. Five equids did not have immunohistochemistry performed on biopsy specimens and therefore were categorized as unclassified lymphoma.

3.3 | Clinical pathology

Clinicopathologic findings at the time of diagnosis are summarized in Table 1. Results were available for 14 of the 15 equids included in the study. Thymidine kinase activity was evaluated in 1 horse and was normal at 1.5 U/L (reference range, <2.7 U/L).[7] Three of 4 horses tested were equine herpes virus-5 (EHV-5) PCR positive on biopsy specimens of neoplastic tissue (2 cutaneous TCRLBCL, 1 multicentric TCRLBCL).

3.4 | Chemotherapy

Chemotherapeutic protocols varied widely, and intervals between chemotherapy administration ranged from 7-30 days between treatments, depending on the drugs included in each protocol. Drugs included in various protocols are summarized in Table 2. Three horses received a 5-drug chemotherapy protocol (1 of which also received corticosteroids): 2 horses received doxorubicin, vincristine, L-asparaginase, lomustine, and cyclophosphamide, and 1 horse received doxorubicin, vincristine, L-asparaginase, cyclophosphamide, and cytosine arabinoside. Four horses received a 4-drug chemotherapy protocol (3 of which also received corticosteroids): 2 horses received doxorubicin, vincristine, L-asparaginase, and cyclophosphamide, and 2 horses received vincristine, L-asparaginase, cyclophosphamide, and lomustine. Four horses, 2 of which were pregnant mares, received a 3-drug chemotherapy protocol (all 4 received corticosteroids): 2 horses

TABLE 1 Clinicopathologic findings at the time of diagnosis for 14 equids treated for lymphoma with chemotherapy, organized by (1) anatomic distribution and (2) subsequent response to treatment

Anatomic distribution	PCV (%)	WBCC (cells/μL)	Neutrophils (cells/μL)	Lymphocytes (cells/μL)	Platelets (/μL)	Total Protein (g/dL)	Fibrinogen (mg/dL)	Albumin (g/dL)	Total calcium (mg/dL)
Multicentric (n = 9)	38 (30-53)	7450 (4930-14 700)	5886 (3451-11 350)	1299 (1000-2800)	134 000 (102 000-150 000)	6.0 (49-7.3)	400 (300-700)	2.81 (2.3-3.2)	11 (10.55-12.7)
Cutaneous (n = 2)	33.5 (28-39)	8320 (5800-10 840)	6650 (3850-9450)	1165 (740-1590)	96 000 (55 000-137 000)	5.3 (4.8-5.8)	624[a]	2.84 (2.8-2.87)	12.2 (12.1-12.27)
Alimentary (n = 3)	29 (26-36)	7400 (4140-7950)	3920 (3360-5400)	2390 (600-3030)	121 000[a]	5.4 (5.1-5.7)	400 (200-500)	2.8 (2.7-2.9)	11.6 (10.9-12.1)

Values listed are medians (range).
Abbreviations: PCV, packed cell volume; WBCC, white blood cell count.
[a] Information from only 1 horse available.

(including 1 pregnant mare) received vincristine, cyclophosphamide, and cytosine arabinoside; 1 horse received doxorubicin, vincristine, and cyclophosphamide; and the second pregnant mare received vincristine, L-asparaginase, and cyclophosphamide. Two equids received a 2-drug chemotherapy protocol (both received corticosteroids): 1 horse received doxorubicin and lomustine, and the donkey received vincristine and cyclophosphamide. Two horses received a 1-drug chemotherapy protocol consisting of lomustine, and both of these horses received corticosteroids.

Vincristine was administered at a dosage of 0.55-0.7 mg/m^2 IV in 12 patients. Doxorubicin was administered IV at a dosage of 70 mg/m^2 in 3 horses, 60 mg/m^2 in 1 horse, 50 mg/m^2 in 2 horses, and 35 mg/m^2 in 1 horse. L-asparaginase was administered SC at a dosage of 10 000 U/m^2 in 8 horses. Cyclophosphamide was administered IV at a dosage ranging from 150 to 800 mg/m^2 in 12 patients. Lomustine was administered at a dose of 65 mg/m^2 intragastrically in 7 horses. Cytosine arabinoside was administered IV at a dosage of 175-250 mg/m^2 in 3 horses.

3.5 | Adjunctive treatments

Twelve patients received corticosteroid treatment during chemotherapy: prednisolone in all 12 equids at a dosage of 0.5-1 mg/kg PO q12-24 hours and dexamethasone in 2 horses at a dosage of 0.05-0.1 mg/kg IV or PO q24h. One horse with cutaneous lymphoma received perioperative intralesional cisplatin injected around surgical margins after removal of a preputial mass, before starting systemic chemotherapy.

Valacyclovir was administered in 3 horses, all of which were positive for EHV-5, at a dosage of 30-40 mg/kg PO q8-12h. Two patients with alimentary lymphoma had small intestinal resections performed via celiotomy.

3.6 | Adverse effects

Nine of 15 equids exhibited a total of 14 adverse effects attributable to chemotherapy. Drugs believed to result in the adverse effects included doxorubicin (n = 4), vincristine (n = 4), cyclophosphamide (n = 2), and L-asparaginase (n = 1), whereas a direct causal relationship with a particular drug could not be determined in 3 adverse effects. Adverse effects were graded according to the Veterinary Cooperative Oncology Group common terminology criteria for adverse event grading system[8] and included grade 1 alopecia (n = 2); grade 1 neutropenia (n = 2); grade 1 lymphopenia (n = 3); grade 1 lethargy (n = 1); grade 2 neurotoxicity (n = 1); grade 2 gastrointestinal signs (colic) (n = 1); grade 1 hypersensitivity, (n = 1); grade 2 hypersensitivity (n = 2); grade 5 hypersensitivity (n = 1). All hypersensitivities were associated with doxorubicin administration (n = 4).

Four of 7 horses that received doxorubicin experienced adverse effects attributed to the drug. All horses were premedicated with flunixin meglumine and diphenhydramine hydrochloride before doxorubicin administration and re-dosed as needed if signs of hypersensitivity were detected, according to previously published administration protocols for this drug.[9] One horse developed tachycardia (76 bpm) 4 hours after and low-grade fever (38.3°C) 20 hours after doxorubicin

TABLE 2 Chemotherapeutic agents included in treatment protocols for 15 equids with lymphoma

Number of equids receiving the combination		Total	2	1	2	2	2[a]	1	1[a]	1	1	2
Drug	Doxorubicin (35-70 mg/ m^2 IV)	7	X	X	X			X		X		
	Vincristine (0.55-0.7 mg/ m^2 IV)	12	X	X	X	X	X	X	X		X	
	L-asparaginase (10 000 U/m^2 SC)	8	X	X	X	X			X			
	Lomustine (CCNU; 65 mg/ m^2 IG)	7	X			X				X		X
	Cyclophosphamide (150-800 mg/ m^2 IV)	12	X	X	X	X	X	X	X		X	
	Cytosine arabinoside (175-250 mg/m^2 IV)	3		X			X					
	Corticosteroids	12	0	1	2	1	2	1	1	1	1	2

[a] Indicates 1 horse treated with protocol was a pregnant mare.

administration, which resolved without treatment (grade 1 hypersensitivity). Another horse also developed tachycardia (72 bpm) and fever (39.4°C) the day of doxorubicin administration, which resolved with 1 additional dose of flunixin meglumine (grade 2 hypersensitivity). One horse developed tachycardia (60 bpm) and fever (39.9°C) 6 hours after doxorubicin administration and was found to have an increased plasma cardiac troponin I concentration (0.81 mg/mL; reference range, 0.00-0.07 ng/mL), hypoproteinemia (5.0 g/dL; reference range, 5.5-7.5 g/dL), and thrombocytopenia (50 000 platelets/μL; reference range, 72 000-183 000/μL) (grade 2 hypersensitivity). In this horse, flunixin meglumine and diphenhydramine were administered, and plasma cardiac troponin I and total protein concentrations improved gradually over the next 3 days, and doxorubicin subsequently was omitted from this horse's chemotherapy protocol. One horse died after doxorubicin administration while in PR to chemotherapy consisting of a 5-drug protocol (grade 5 hypersensitivity). This horse previously had received doxorubicin without problems and was normal before and during drug infusion. This horse developed tachycardia (80 bpm) and fever (39.3°C) beginning 2 hours after doxorubicin administration, progressing to tachypnea and pulmonary edema, with subsequent death 18 hours post-administration. Hematology of this horse disclosed hemoconcentration (hematocrit, 67.4%), lymphopenia (1000 lymphocytes/μL), hypoglycemia (49 mg/dL), azotemia (4.5 mg/dL), and hyperlactatemia (>12 mmol/L). Necropsy did not identify an obvious cause of death, and cardiac histopathology was unremarkable.

One horse developed lethargy and pelvic limb edema at an escalating cyclophosphamide dosage of 800 mg/m^2, which resolved without treatment and was not noted at lower dosages (grade 1). One horse developed mild colic, decreased manure production, and inappetence 6 hours after L-asparaginase administration (grade 2 gastrointestinal). One horse developed focal seizure-like activity after chemotherapy with vincristine and cyclophosphamide (grade 2 neurotoxicity); seizures were controlled with PO phenobarbital in this horse, and a direct causal association could not be determined. Two horses experienced partial hair loss of the mane and tail during multidrug chemotherapy (grade 1 alopecia).

Three horses developed mild (grade 1) hematologic adverse effects during chemotherapy noted on screening CBCs before administration of subsequent chemotherapy; this followed vincristine as the most recently administered medication in 2 horses, and in 1 horse the inciting drug was not recorded. Three horses had lymphopenia, with lymphocyte counts of 1050/μL, 1050/μL, and 1000/μL. Two of the same 3 horses also had concurrent neutropenia with neutrophil counts of 1670/μL and 2070/μL.

Two horses experienced complications not considered to be true drug adverse effects. One horse developed bacterial thrombophlebitis at the jugular vein catheter site used for cyclophosphamide administration, with subsequent development of head swelling that resolved with antimicrobial administration. One horse (the stallion) was castrated to remove the lymphoma-affected testicles 14 days before starting chemotherapy and developed a castration site infection 8 days after castration. The horse was not leukopenic on CBC performed 3 days before or 3 days after castration.

3.7 | Outcome

Treatment response and survival time are summarized in Table 3. The Kaplan-Meier survival curve is shown in Figure 1.

Complete remission was achieved in 5 horses (33.3%), PR was achieved in 9 equids (60%), and SD was achieved in 1 horse. Two of 3 horses with EHV-5-associated lymphoma achieved CR and the third achieved PR. Overall response rate was 93.3% (14/15). Four of the 5 horses that initially achieved CR experienced disease relapse at a median of 9.25 months (range, 4-41 months).

Kaplan-Meier median estimated survival time was 590 days (95% CI, 107-1073 days; Figure 1). Overall median survival time was 8 months (range, 1-46 months). Median survival time for multicentric lymphoma was 7 months (range, 1-32 months), median survival time for alimentary lymphoma was 7 months (range, 2-36 months), and median survival time for cutaneous lymphoma was 34 months (range, 21-46 months). Median survival time for horses that achieved CR was 21 months (range, 4-46 months), whereas median survival time for patients that achieved PR was 7 months (range, 1-36 months). Table 3 reports median survival time for patients with different treatment protocols.

Ten of 13 (77%) equids, for which long-term follow-up information was available, died or were euthanized. Three horses are alive at the time of writing. Of these, 1 is in PR and 2 are in CR. Of the 2 horses still in CR, 1 is in second CR after a recurrence of lymphoma at 41 months after initial diagnosis (36 months after completion of previous chemotherapy consisting of lomustine only), which was treated with lomustine, and is currently still alive 5 months after recurrence (46 months after initial diagnosis). Two horses were lost to follow-up (1 in PR and 1 in SD at last contact at 8 and 28 months, respectively).

TABLE 3 Long-term outcome after chemotherapy for lymphoma in 15 equids

	Complete remission (n = 5)	Partial response (n = 9)	Stable disease (n = l)	Median survival time in months (range)[a]
Breed				
Warmblood (n = 6)	3	2	1	21.25 (4-34)
Quarter Horse (n = 3)	1	2	...	8 (7-46)
Standardbred (n = 2)	...	2	...	1, 7
Arabian (n = l)	...	1	...	36
Thoroughbred (n = l)	1	...	...	21
Tennessee Walker (n = l)	...	1	...	2.5
Donkey (n = l)		1	...	2
Anatomic distribution				
Multicentric (n = 9)	2	6	1	7 (1–32)
Cutaneous (n = 3)	3	...	...	34 (21–46)
Alimentary (n = 3)	...	3	...	7 (2-36)
Immunophenotype				
TCRLBCL (n = 6)	2	4	...	14.5 (1–34)
Large B cell (n = 2)	1	...	1	4, 28
T cell (n = 2)	2	...	...	19.5, 46
Unclassified (n = 5)	...	5	...	7 (2–36)
EHV5 positive (n = 3)	2	1	...	32 (21-34)
Chemotherapy				
5 drug protocol (n = 3)	1	1	1	21 (5-28)
4 drug protocol (n = 4)	2	2	...	33 (4–36)
3 drug protocol (n = 4)	1	3	...	4.75 (1-19.5)
2 drug protocol (n = 2)	...	2	...	2, 8
1 drug (lomustine) protocol (n = 2)	1	1	...	7, 46

Abbreviations: EHV, equine herpes virus; TCRLBCL, T-cell rich large B-cell lymphoma.
[a] If greater than 2 horses, median survival is reported, if less than or equal to 2 horses, survival lengths of individual horse(s) are listed.

Eight equids were euthanized. Of these, 2 horses and 1 donkey with alimentary lymphoma were euthanized at 2, 2.5, and 7 months after diagnosis because of progression of their clinical signs while still on chemotherapy, after initially achieving PR. Another 2 horses were euthanized at 7 and 36 months after diagnosis because of lymphoma relapse while no longer receiving chemotherapy, after initially achieving PR. Of these 2, 1 was a pregnant mare that survived to foaling in PR and gave birth to a small but otherwise normal foal, but was euthanized 2 months after foaling (7 months after lymphoma diagnosis) because of lymphoma relapse and associated weight loss while no longer receiving chemotherapy. One horse experienced disease relapse at 19.5 months after diagnosis while no longer receiving chemotherapy after initially achieving CR and was euthanized at that time. One horse experienced disease relapse 4 months after diagnosis while still receiving chemotherapy, after initially achieving CR, and was euthanized at that time. One horse was euthanized 21 months after diagnosis of lymphoma because of a stifle injury while no longer receiving chemotherapy and still in CR.

Two horses died. One died 5 months after diagnosis, while in PR, after doxorubicin administration. One pregnant mare died 1 month after diagnosis because of progression of a mediastinal mass and pleural effusion while still receiving chemotherapy, after initially achieving PR.

4 | DISCUSSION

In this retrospective study, we report the survival times and incidence of adverse effects after chemotherapy for lymphoma in 15 equids. Horses with cutaneous lymphoma and those that achieved CR tended to have longer median survival times than those with alimentary or multicentric lymphoma and those that achieved PR. Adverse effects were common (occurring in two-thirds of cases) but were usually mild and self-limiting, although 1 horse died as a consequence of adverse effects attributed to doxorubicin administration. These findings support the use of chemotherapy for the treatment of lymphoma in horses and expand the body of information available regarding potential adverse effects.

Lymphoma is a common malignant neoplasm in horses, with a prevalence of 3% based on necropsy in 241 mature and geriatric horses in a recent study.[2,10] Lymphoma in horses most commonly is classified as TCRLBCL, and it was the most prevalent form in our study, affecting 6 of 15 equids, consistent with previous reports.[2] Common clinical findings in horses with lymphoma have been described previously,[11–13] and were not the focus of our retrospective study. Anemia and thrombocytopenia often are reported but were fairly uncommon in our case series. Thymidine kinase recently was reported to be a useful indicator of lymphoma in horses.[7] Only 1 horse in our study had this analyte measured, and it was found to be within normal limits.

In our study, horses with cutaneous lymphoma all achieved CR and tended to have longer survival times than did horses with multicentric or alimentary lymphoma (median of 34 months versus 7 months), suggesting that anatomic distribution may affect prognosis. In dogs with lymphoma, the strongest prognostic factors include grade, immunophenotype, stage (anatomic distribution and extent), and substage.[14] The effect of anatomic distribution is such that most extranodal lymphomas in dogs, such as gastrointestinal and cutaneous forms, are associated with a worse prognosis than multicentric lymphoma.[14] The worse prognosis in dogs with cutaneous lymphoma compared to horses with cutaneous lymphoma likely relates to immunophenotype, as most cutaneous lymphomas in dogs are T-cell lymphoma, whereas most cutaneous lymphomas in horses are TCRLBCL. In dogs with lymphoma, most T-cell neoplasms have shorter remission and survival times than do B-cell lymphomas. T-cell lymphoma, however, now is understood to represent a large group of different subtypes, with variable prognosis, dependent on subtype.[14] Although only 2 horses in our study had T-cell lymphoma, these horses were among those with the longest survival times. One of these horses had cutaneous lymphoma, whereas the other had multicentric lymphoma. It would have been of interest to further subclassify the T-cell lymphoma in these horses; however, further subtyping was not available.

Because of the retrospective nature of our study, the optimal chemotherapeutic approach for horses with lymphoma cannot be determined because of the lack of randomization and insufficient number of cases for statistical evaluation. However, when examining

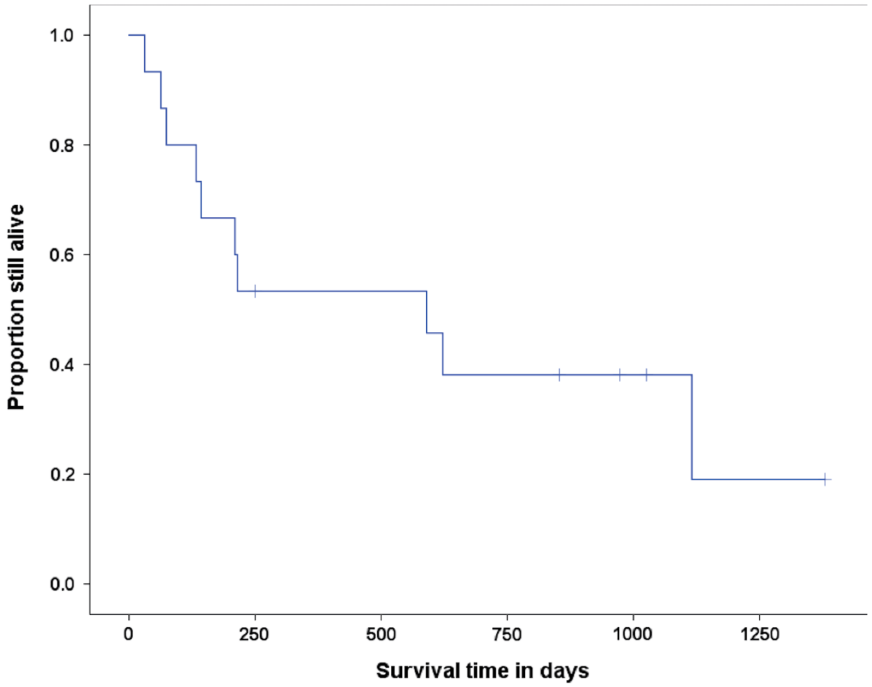

FIGURE 1 Kaplan-Meier plot of survival time for 15 equids treated for lymphoma with chemotherapy

median duration of survival for patients that received different chemotherapy regimens, the horses that received 5- and 4-drug protocols had longer median survival time than did patients that received all other regimens except the 1-drug (lomustine) protocol. This finding is mostly concordant with results in dogs with lymphoma, in which multiagent chemotherapeutic protocols generally are considered superior to single-agent protocols because of decreased potential for development of drug resistance.[14] Single-agent chemotherapy usually is not expected to be superior in treatment of lymphoma in dogs. Lomustine is the single-agent drug of choice in dogs with epitheliotropic T-cell lymphoma, and a recent study of epitheliotropic lymphoma in dogs found no association between the type of chemotherapy treatment (multiagent protocol vs lomustine alone) and survival time.[15] Therefore, the apparent advantage of the 1-drug regimen may represent true superiority of this drug in horses with lymphoma although this is considered less likely. Alternatively, this result may be affected by selection bias, because only 2 horses received this treatment, and they may have been less severely affected. Of the 2 horses that received a 1-drug protocol, 1 had T-cell lymphoma whereas the other was unclassified. Therefore, the effect of immunophenotype on the apparent advantage of the 1-drug protocol is difficult to determine. Thus, our results do not clearly determine whether multiagent or single-agent protocols are superior for treatment of lymphoma in horses.

Corticosteroids alone may result in remission in some lymphomas, and the corticosteroid administration in 12 of the 15 horses in our study complicates interpretation of the benefits of chemotherapy. However, a study evaluating first-line multidrug protocols for treatment of multicentric lymphoma found no effect of prednisolone on treatment outcome in 81 dogs.[16] Because of small case numbers and substantial variation in treatment protocols, the impact of corticosteroid administration on treatment of horses with lymphoma was not clearly evaluated in our study. Further studies will be required to clarify the effect of corticosteroids as compared to chemotherapy in horses with lymphoma.

Adverse effects were seen in almost two-thirds of the horses in our study, most notably after doxorubicin administration. Doxorubicin has been studied extensively, with safety and dose escalation studies published.[9,17] Four of 7 horses that received doxorubicin in our study experienced adverse effects after doxorubicin, with 1 horse dying as a consequence of doxorubicin toxicity, despite administration of nonsteroidal anti-inflammatory medications and antihistamines. Doxorubicin is an anthracycline antibiotic that is known to be both acutely and cumulatively cardiotoxic.[18] In a phase I dose escalation study to determine dose-limiting toxicosis and maximum tolerated dose in 17 horses, no death was noted.[17] Maximum tolerated dosage was 75 mg/m^2, and neutropenia and hypersensitivity reactions were the most common dose-limiting toxicoses seen.[17] Hypersensitivity reactions to chemotherapy are defined as unexpected reactions to drug administration with signs not consistent with the drug's known toxicity.[19] The adverse reactions seen in the horses in our study were consistent with hypersensitivity reactions seen with chemotherapy in other species,[20] and it is likely that the death of 1 horse after doxorubicin administration was a result of a hypersensitivity reaction. Hypersensitivity reactions to doxorubicin described in humans include edema, dyspnea, and anaphylaxis,[19] and complement activation is believed to be involved in the pathogenesis.[21]

Reported toxic effects of cyclophosphamide, an alkylating agent, in other species include myelosuppression, hemorrhagic cystitis, and cardiotoxicity,[22] none of which were noted in the horses reported here. One horse in our study developed an adverse reaction to cyclophosphamide characterized by lethargy and limb edema at a dosage of 800 mg/m^2. This dosage is much higher than what has been reported previously in horses (200 mg/m^2).[23] Cyclophosphamide is commonly included in chemotherapeutic protocols for horses with neoplasia,

including lymphoma. Dosages regularly used in chemotherapeutic protocols for horses are extrapolated from other species, and cyclophosphamide dose optimization has not been reported in horses. Dose intensity refers to the dose of active drug over time, and multiple studies in humans and animals with cancer have shown that increasing chemotherapeutic dose intensity correlates with increased antitumor effect.[24,25] Chemotherapeutic drug dose escalation is a strategy of gradual dose increases with careful monitoring for toxicity, utilized to determine the highest tolerated dose and thereby achieve higher dose intensity and maximum efficacy. Three of the horses in our study received escalating doses of cyclophosphamide to maximum doses of 310 mg/m^2 IV, 450 mg/m^2 IV, and 800 mg/m^2 IV. These findings suggest that cyclophosphamide dose escalation may be used in horses to achieve higher chemotherapeutic dose intensity while minimizing adverse effects, and that the appropriate therapeutic dose of cyclophosphamide in horses may be higher than previously used. Further studies are warranted to determine the optimal dosage of cyclophosphamide for horses. Dose escalation of other chemotherapeutic agents was not performed in any of the horses in our study, and the effect of dose escalation of cyclophosphamide on outcome was not evaluated.

Two pregnant mares were treated using chemotherapy in our study. Importantly, both mares were at a stage in gestation beyond fetal organogenesis at the initiation of chemotherapy. Vincristine, a plant alkaloid, is believed to be less teratogenic than other chemotherapeutic agents,[26,27] and for this reason, it was used in both pregnant mares. Although only 2 pregnant mares were included in our study, and only 1 of those mares survived to foaling, our study provides additional information regarding the use of chemotherapy for treatment of lymphoma during pregnancy in horses.

Although chemotherapeutic protocols have been reported in horses with lymphoma,[3,4,23,28] these reports have been limited to case reports and case series, and little information is available on the efficacy, safety, and outcome of chemotherapy in horses. Chemotherapy in our study was effective, with 14 equids achieving CR or PR after treatment and 7 horses surviving >1 year after diagnosis. Unfortunately, lymphoma in horses remains a malignant neoplasm with poor long-term prognosis. Four of 5 horses that initially achieved CR subsequently experienced disease relapse, although 1 of the 4 achieved and remains in a second CR; 10 of the 13 equids in our study, for which long-term follow-up was available, died or were euthanized during or after chemotherapy for lymphoma.

Many barriers exist to implementing chemotherapy in horses. These include financial constraints, availability of the drugs used, and personnel exposure and environmental contamination with chemotherapeutic drug residue. In addition, owner concerns exist regarding performance of horses while receiving chemotherapy as well as impact on fertility. A number of the horses in our study continued to be exercised during their courses of chemotherapy, including at least 2 horses that successfully competed during treatment, suggesting carefully planned chemotherapy need not necessarily impact performance beyond any effects related to the disease process itself.

Our study is limited by its retrospective nature. Retrospective studies inherently suffer from information bias and lack of complete medical records. In addition, case numbers were small and included different subtypes of lymphoma, staging was inconsistent, and no control population was utilized to compare overall survival of horses that received chemotherapy with horses not treated by chemotherapy. Ideally, a randomized controlled clinical trial should be used to evaluate the efficacy of chemotherapy for lymphoma in horses. Nonetheless, ours is the largest case series to date of horses with lymphoma treated using chemotherapy.

In conclusion, chemotherapy may be used successfully for the treatment of horses with lymphoma to achieve remission and potentially increase survival time, with results likely dependent on anatomic distribution and stage of disease. Adverse effects associated with chemotherapy are common but usually mild and self-limiting.

CONFLICT OF INTEREST DECLARATION

Authors declare no conflict of interest.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Authors declare no IACUC or other approval was needed.

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Daniela Luethy https://orcid.org/0000-0003-1693-2147
Daniela Bedenice https://orcid.org/0000-0001-8451-8306
Lauren Proctor-Brown https://orcid.org/0000-0002-6991-1609
Joy E. Tomlinson https://orcid.org/0000-0001-7365-3967
Amy L. Johnson https://orcid.org/0000-0003-2507-0040

REFERENCES

1. Knowles EJ, Tremaine WH, Pearson GR, Mair TS. A database survey of equine tumours in the United Kingdom. *Equine Vet J*. 2016;48(3):280-284.
2. Durham AC, Pillitteri CA, San Myint M, Valli VE. Two hundred three cases of equine lymphoma classified according to the World Health Organization (WHO) classification criteria. *Vet Pathol*. 2013;50(1):86-93.
3. Saulez MN, Schlipf JW, Cebra CK, McDonough SP, Bird KE. Use of chemotherapy for treatment of a mixed-cell thoracic lymphoma in a horse. *J Am Vet Med Assoc*. 2004;224(5):733-738. 699.
4. Doyle AJ, MacDonald VS, Bourque A. Use of lomustine (CCNU) in a case of cutaneous equine lymphoma. *Can Vet J*. 2013;54(12):1137-1141.
5. Vail DM, Michels GM, Khanna C, Selting KA, London CA. Response evaluation criteria for peripheral nodal lymphoma in dogs (v1.0)—a Veterinary Cooperative Oncology Group (VCOG) consensus document. *Vet Comp Oncol*. 2010;8(1):28-37.
6. Byrne BA, Yvorchuk-St. Jean K, Couto CG, Kohn CW. Successful management of lymphoproliferative disease in two pregnant mares. Proceedings of the 11th Annual Conference Veterinary Cancer Society. 1991;8-9.

7. Larsdotter S, Nostell K, von Euler H. Serum thymidine kinase activity in clinically healthy and diseased horses: a potential marker for lymphoma. *Vet J*. 2015;205(2):313-316.
8. No authors listed. Veterinary cooperative oncology group—common terminology criteria for adverse events (VCOG-CTCAE) following chemotherapy or biological antineoplastic therapy in dogs and cats v1.1. *Vet Comp Oncol*. 2016;14(4, 4):417-446.
9. Théon AP, Pusterla N, Magdesian KG, Wittenburg L, Marmulak T, Wilson WD. A pilot phase II study of the efficacy and biosafety of doxorubicin chemotherapy in tumor-bearing equidae. *J Vet Intern Med*. 2013;27(6):1581-1588.
10. Miller MA, Moore GE, Bertin FR, Kritchevsky JE. What's new in old horses? Postmortem diagnoses in mature and aged Equids. *Vet Pathol*. 2016;53(2, 2):390-398.
11. van Den HR, Franken P. Clinical aspects of lymphosarcoma in the horse: a clinical report of 16 cases. *Equine Vet J*. 1983;15(1):49-53.
12. Meyer J, Delay J, Bienzle D. Clinical, laboratory, and histopathologic features of equine lymphoma. *Vet Pathol*. 2006;43(6):914-924.
13. Miller CA, Durham AC, Schaffer PA, Ehrhart EJ, Powers BE, Duncan CG. Classification and clinical features in 88 cases of equine cutaneous lymphoma. *J Vet Diagn Invest*. 2015;27(1):86-91.
14. Zandvliet M. Canine lymphoma: a review. *Vet Q*. 2016;36(2):76-104.
15. Chan CM, Frimberger AE, Moore AS. Clinical outcome and prognosis of dogs with histopathological features consistent with epitheliotropic lymphoma: a retrospective study of 148 cases (2003-2015). *Vet Dermatol*. 2018;29(2):154-e59.
16. Zandvliet M, Rutteman GR, Teske E. Prednisolone inclusion in a first-line multidrug cytostatic protocol for the treatment of canine lymphoma does not affect therapy results. *Vet J*. 2013;197(3):656-661.
17. Théon AP, Pusterla N, Magdesian KG, Wilson WD. Phase I dose escalation of doxorubicin chemotherapy in tumor-bearing equidae. *J Vet Intern Med*. 2013;27(5):1209-1217.
18. Gianni L, Grasselli G, Cresta S, Locatelli A, Viganò L, Minotti G. Anthracyclines. *Cancer Chemother Biol Response Modif*. 2003;21:29-40.
19. Syrigou E, Makrilia N, Koti I, Saif MW, Syrigos KN. Hypersensitivity reactions to antineoplastic agents: an overview. *Anticancer Drugs*. 2009;20(1):1-6.
20. Blake MK, Carr BJ, Mauldin GE. Hypersensitivity reactions associated with L-asparaginase administration in 142 dogs and 68 cats with lymphoid malignancies: 2007–2012. *Can Vet J*. 2016;57(2):176-182.
21. Chanan-Khan A, Szebeni J, Savay S, et al. Complement activation following first exposure to pegylated liposomal doxorubicin (Doxil): possible role in hypersensitivity reactions. *Ann Oncol*. 2003;14(9):1430-1437.
22. Fraiser LH, Kanekal S, Kehrer JP. Cyclophosphamide toxicity. Characterising and avoiding the problem. *Drugs*. 1991;42(5):781-795.
23. Vander Werf K, Davis E. Disease remission in a horse with EHV-5-associated lymphoma. *J Vet Intern Med*. 2013;27(2, 2):387-389.
24. Azim HA, Santoro L, Bociek RG, Gandini S, Malek RA, Azim HA. High dose intensity doxorubicin in aggressive non-Hodgkin's lymphoma: a literature-based meta-analysis. *Ann Oncol*. 2010;21(5):1064-1071.
25. Ma X, Xu Y, Zhang W, et al. High-intensity chemotherapy is associated with better prognosis in young patients with high-risk diffuse large B-cell lymphoma: a 10-year single-center retrospective cohort study. *Med Sci Monit*. 2016;22:1792-1800.
26. Autio K, Rassnick KM, Bedford-Guaus SJ. Chemotherapy during pregnancy: a review of the literature. *Vet Comp Oncol*. 2007;5(2):61-75.
27. Azim HA, Pavlidis N, Peccatori FA. Treatment of the pregnant mother with cancer: a systematic review on the use of cytotoxic, endocrine, targeted agents and immunotherapy during pregnancy. Part II: hematological tumors. *Cancer Treat Rev*. 2010;36(2):110-121.
28. McGovern KF, Lascola KM, Davis E, Fredrickson RL, Tan R. T-cell lymphoma with immune-mediated anemia and thrombocytopenia in a horse. *J Vet Intern Med*. 2011;25(5):1181-1185.

How to cite this article: Luethy D, Frimberger AE, Bedenice D, et al. Retrospective evaluation of clinical outcome after chemotherapy for lymphoma in 15 equids (1991-2017). *J Vet Intern Med*. 2019;33:953–960. https://doi.org/10.1111/jvim.15411