Accuracy of ultrasonography to detect hepatic and splenic lymphomatous infiltration in dogs and cats

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STRUCTURED SUMMARY

Objectives: To determine the sensitivity, specificity and accuracy of ultrasonography in the detection of lymphomatous infiltration of the liver and spleen in a large population of dogs and cats with lymphoma. The second aim of this study is to determine if specific ultrasonographic features of the liver and spleen in dogs are associated with lymphomatous infiltration or a specific immunophenotype of multicentric lymphoma.

Methods: Blinded retrospective review of ultrasonographic images of the liver and/or spleen in 132 dogs and 29 cats with cytologically or histologically confirmed lymphoma by two board-certified veterinary radiologists.

Results: Ultrasonography had a sensitivity, specificity, accuracy, positive predictive value and negative predictive value of 16.7%, 91.0%, 55.9%, 62.5% and 55.0% for the detection of lymphomatous infiltration of the liver, and 73.1%, 93.9%, 82.6%, 93.4% and 74.7% for the spleen. In dogs, an ultrasonographically normal liver was statistically associated with not having lymphomatous infiltration, leopard-spotted splenic parenchyma and splenomegaly were independently statistically associated with lymphomatous infiltration and leopard-spotted splenic parenchyma was also statistically associated with the B-cell immunophenotype of multicentric lymphoma.

Clinical significance: Ultrasonography of the spleen and liver is specific but not sensitive in the detection of lymphomatous infiltration. A leopard spotted splenic parenchyma in dogs is highly specific for lymphomatous infiltration and can also be used to predict immunophenotype of multicentric lymphoma.

INTRODUCTION

Lymphoma is a commonly diagnosed neoplasm both in dogs (Dobson *et al.* 2002) and cats (Manuali *et al.* 2020). Complete staging in the initial phase can provide valuable information to allow assessment of the organs affected by lymphoma at diagnosis, provide an indication of prognosis and allow assessment of remission status after treatment (Marconato 2011). Based on the World Health Organisation classification system for lymphoma in domestic animals (Vail *et al* 2013), it is necessary to determine involvement of lymph nodes, liver, spleen and other organs. In the liver and spleen, this can be achieved with either fine needle aspiration or needle core biopsy. Fine needle aspiration is generally preferred in the first instance, as it precludes the need for anaesthesia, involves less risk and can penetrate deeper if needed (Watson *et al* 2011, Nerschbach *et al* 2016). Fine needle aspiration has been shown to be as valuable as needle core biopsy for diagnosing splenic neoplasia in the dog (Watson *et al* 2011). Obtaining samples may not always be possible due to concerns over increased risk of severe bleeding (due to thrombocytopenia or prolonged coagulation assay times) (Bigge *et al.* 2001), operator skills or financial constraints.

Previous reports show conflicting evidence on the utility of ultrasonography (US) for detecting lymphomatous infiltration of the liver(Nyland 1984, Lamb *et al.* 1991, Crabtree *et al.* 2010, Warren-Smith *et al.* 2012), but this modality appears to be more sensitive for the detection of infiltration in the spleen than in the liver (Lamb *et al.* 1991, Crabtree *et al.* 2010). The studies performed to determine the value of US and the correlation of specific features with the cytological diagnosis of lymphoma have been hampered by their relatively small case numbers and in some cases by less modern equipment (Nyland 1984, Lamb *et al.* 1991, Crabtree *et al.* 2010, Warren-Smith *et al.* 2012).

The literature describes a variety of ultrasonographic appearances of lymphomatous infiltration of the liver including diffuse changes in echogenicity or discrete nodules, which can be hypoechoic, have a target appearance or be cavitary (Nyland 1984, Lamb *et al.* 1991, Voros *et al.* 1991, Crabtree *et al.* 2010, Warren-Smith *et al.* 2012). Ultrasonographic changes consistent with lymphomatous infiltration of the spleen have been described as complex or cavitated masses or areas of decreased echogenicity, which can be manifested as either well-defined nodules, ill-defined areas or leopard-spotted appearance (which is also referred to as moth-eaten, Swiss-cheese or honeycomb patterns) (Lamb *et al.* 1991, Crabtree *et al.* 2010, Bertal *et al.* 2018, Harel *et al.* 2020).

The purpose of this study is to determine the sensitivity, specificity, and accuracy of US in the detection of lymphomatous infiltration of the liver and spleen in a large population of dogs and cats. The second aim of this study is to determine if specific ultrasonographic features of these organs in dogs are more likely to be correlated with lymphomatous infiltration or the immunophenotype of multicentric lymphoma than others.

METHODS

An electronic database search of PubMed was performed in November 2020, with the keywords “lymphoma”, “ultrasonography”, “dogs” and “cats”. The references of all appropriate studies were assessed to identify further reports. The following textbook has also been consulted: Small Animal Clinical Oncology, 5th edition.

Ethical approval was granted by the XXX at the XXX. The US database of the XXX, was searched for all cases with a diagnosis of lymphoma between November 2010 and November 2019. Medical records of these dogs and cats were reviewed and cases were included if they had a diagnosis of lymphoma confirmed on cytology and/or histopathology and had undergone abdominal US contemporaneously with the diagnosis. In 17/161 cases immunohistochemistry, flow cytometry or PARR were used to confirm the diagnosis when cytology or histology were suggestive but not conclusive. Cases were included if they had diagnostic quality ultrasound images of the liver and/or the spleen available at initial presentation, had an ultrasound-guided fine needle aspirate of the liver and/or spleen taken at the same time of image acquisition and had consequential cytological analysis of the aspirate. Cases were excluded if there was excessive motion blur on the ultrasound images or if it was not possible to accurately assess the liver and/or spleen for the different classification features. Additionally, cases were excluded if they had received any corticosteroid or chemotherapeutic agent in the 30 days prior to abdominal US or if a detailed history prior to their diagnosis was not available. Data collected regarding each animal included signalment, weight, cytological or histopathological diagnosis, anatomic form and immunophenotype of lymphoma when available.

Ultrasound examinations were performed by or under supervision of a board-certified radiologist, with animals in right lateral recumbency using a RS80A (Samsung Healthcare) with a 4-9MHz microconvex or 3-12MHz linear probe, Logiq S7 (General Electric Medical System) with a 7-10MHz microconvex or 8-15MHz linear probe, Logiq 7 (General Electric Medical System) with a 3.5-11.5MHz microconvex or 4.5-13MHz linear probe or Z.one 1 (Zonare Medical Systems) with a 4-9MHz microconvex or 5-14MHz linear probe depending on the machine which was in clinical use at the time. All images were stored in Digital Imaging and Communications in Medicine (DICOM) files. Fine needle aspirates of the liver and spleen were obtained using a 22G hypodermic needle with a 5ml syringe attached, without aspiration. Images of the liver and spleen were independently reviewed by two board-certified radiologists, who did not have access to the cytological results. Images of the liver (Figure 1) were classified as: normal, hepatomegaly, hypoechoic parenchyma, hyperechoic parenchyma, finely heterogeneous parenchyma, strongly heterogeneous, presence of nodules (<2cm), presence of target lesions and presence of masses (>2cm). Images of the spleen (Figure 2) were classified as: normal, splenomegaly, hypoechoic parenchyma, hyperechoic parenchyma, finely heterogeneous parenchyma, leopard-spotted parenchyma, presence of nodules (<2cm), presence of target lesions and presence of masses (>2cm). The liver and spleen of each case were allowed to have more than one characteristic. The liver and spleen were then given a grade as to how likely the reviewer thought lymphomatous infiltration of the organ was (0 = no suspicion, 1 = low suspicion, 2 = moderate suspicion, 3 = high suspicion). A consensus on the classification of the ultrasonographic images for all cases was then made by the two radiologists, in one sitting. To assess the performance of ultrasound for the detection of lymphomatous infiltration, the consensus grading for ultrasound infiltration assigned by the reviewers was converted as follows: grades 0-1 were considered negative for infiltration and grades 2-3 were considered positive for infiltration. All cytology samples were evaluated under the supervision of a board-certified pathologist.

All statistical analyses were conducted with the statistical software packages SPSS 25.0 (SPSS Inc, Chicago, Illinois, USA) and R (R version 3.2.0, The R Foundation for Statistical Computing). Descriptive statistics were calculated for variables where necessary; continuous data were summarised as means with standard deviations if normally distributed or median values with interquartile ranges (IQR) if non-normal. Categorical data were summarised as frequencies including 95% confidence intervals (95% CI) if appropriate. Distribution of continuous variables (age and weight) were assessed graphically and tested with the Kolmogorov-Smirnov test. Categorical variables with many categories and/or categories containing only small numbers were examined and categories were collapsed into larger groupings if required.

Sensitivity, specificity, accuracy, positive predictive value, negatively predictive value and their corresponding 95% CI were then calculated as previously described (Newcombe, Robert G. "Two-Sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods," Statistics in Medicine, 17, 857-872 (1998). The consensus grading for ultrasound infiltration was also used to construct receiver operator characteristic (ROC) curves with calculation of their area under curve (AUC) values. Agreement between reviewers for grading categories was determined by calculating their intra-class correlation coefficients (ICC) with corresponding 95% CIs (2-way single measure for absolute agreement).

Independent variables for analysis were generated from the recorded ultrasound findings. Three outcomes were separately considered: 1) ultrasound findings of the liver associated with the presence or absence of hepatic lymphomatous infiltration; 2) ultrasound findings of the spleen associated with the presence or absence of splenic lymphomatous infiltration and 3) ultrasound findings associated with either T- or B-cell multicentric lymphoma. Potential associations between the outcomes and independent variables were examined with binary logistic regression. Cats were excluded from the logistic regression analyses as they were felt to represent a different population and there were too few cases to justify a separate analysis. Variables demonstrating some association on univariable analysis (*P*-value <0.2) were selected for inclusion into a final multivariable model for the outcome concerned. For any paired variables showing correlation (correlation coefficient <0.7), only the variable with the smallest *P*-value was considered for incorporation into the multivariable analysis. The final multivariable models were constructed using a manual backwards stepwise procedure with retention of variables with Wald *P*-values <0.05. First order interaction terms were tested for variables retained in the final models.

RESULTS

A total of 161 animals met the inclusion criteria, comprising 132 dogs and 29 cats. The most common dog breeds were spaniel (n=18), crossbreed (n=14), Border Collie (n=13) and Labrador Retriever (n=10), whilst most common cat breeds were domestic short or long hair (n=21). Most animals were male neutered (58 dogs and 18 cats), followed by female neutered (47 dogs and eight cats), male entire (24 dogs and one cat) and female entire (three dogs and two cats). The median age was 8 years 1 month old for the dogs and 9 years 11 months old for the cats. The median weight was 21.8kg for the dogs and 4.3kg for the cats. Ninety-eight dogs (74.2%) and 4 cats (13.8%) had multicentric lymphoma, 7 dogs (5.3%) and 13 cats (44.8%) had gastro-intestinal lymphoma, 16 dogs (12.1%) and 3 cats (10.3%) had cutaneous lymphoma, 2 dogs (1.5%) and 1 cat (3.4%) had mediastinal lymphoma and 9 dogs (6.8%) and 8 cats (27.6%) had other anatomic forms of lymphoma. Of the cases that had the immunophenotype determined, 65 dogs and 6 cats had B-cell lymphoma (of which 58 dogs and 1 cat had multicentric lymphoma) and 34 dogs and 4 cats had T-cell lymphoma (of which 18 dogs had multicentric lymphoma).

Within the canine population, every type of ultrasonographic pattern listed above to describe the liver and spleen was identified. Within the feline population, target lesions (in both the liver and spleen), masses over 2cm (in both the liver and spleen), splenic hypoechogenicity and splenic hyperechogenicity were not seen. Agreement between the observers, for both dogs and cats, using intraclass correlation coefficient was 0.924 for the liver and 0.885 for the spleen. One hundred and thirty-four hepatic samples were obtained and of these, six were inconclusive. Four had low cellularity and it was not possible to tell if there was early infiltration or blood derived atypical cells within the other two. One hundred and fifty splenic samples were obtained and of these, four were suggestive of lymphoma but not conclusive and one sample had low cellularity.

Of the 57 dogs that had confirmed hepatic lymphoma, the most common ultrasonographic features included finely heterogeneous parenchyma (57.9%), hepatomegaly (43.8%) and nodules (26.3%) (Table 1). It was common to have more than one ultrasonographic feature, and none of the dogs with confirmed hepatic lymphoma had target lesions within the hepatic parenchyma. Of the 71 dogs that had confirmed splenic lymphoma, the most common ultrasonographic features included leopard-spotted parenchyma (62.0%), splenomegaly (56.3%), nodules (31.0%) and finely heterogeneous parenchyma (29.6%). More than one ultrasonographic feature was commonly seen, and none of the dogs with confirmed splenic lymphoma had a spleen considered normal on ultrasound. Of the three cats that had confirmed hepatic lymphoma, the ultrasonographic features included normal liver, hepatomegaly, hypoechoic parenchyma, finely heterogeneous parenchyma and nodules. None of the cats with confirmed hepatic lymphoma had hyperechoic parenchyma or strongly heterogeneous parenchyma. Of the seven cats that had confirmed splenic lymphoma, the ultrasonographic features included a normal spleen, splenomegaly, finely heterogeneous parenchyma, leopard-spotted parenchyma and nodules.

For the detection of lymphomatous infiltration in the liver for dogs and cats (Table 2), US had a sensitivity of 16.7%, specificity of 91.0% and accuracy of 55.9%. For the detection of lymphomatous infiltration in the spleen for dogs and cats, US had a sensitivity of 73.1%, specificity of 93.9% and accuracy of 82.6%. When dogs were analysed individually, US had a sensitivity, specificity and accuracy of 15.7%, 91.1% and 49.0% for the detection of lymphomatous infiltration in the liver and 77.5%, 91.6% and 83.2% for the spleen. When cats were analysed individually, US had a sensitivity, specificity and accuracy of 33.3%, 90.9% and 84.0% for the detection of lymphomatous infiltration in the liver and 28.6%, 100% and 80.0% for the spleen.

Univariable analysis in dogs indicated that the odds of having a positive diagnosis of lymphoma in the liver in dogs were 4.23 times greater with the presence of masses (p=0.196, 95% CI:0.48-37.54), 3.51 times greater with hypoechoic parenchyma (p=0.125, 95% CI:0.71-17.45), 2.54 times greater with hepatic nodules (p=0.078, 95% CI 0.90-7.14) and 2.26 times greater with finely heterogeneous hepatic parenchyma (p=0.045, 95% CI:1.02-5.04). The grade given by the observers as to the likelihood of lymphomatous infiltration in the liver (p=0.067) was 3.95 times more likely to be correlated with a positive result for grade 1 (95% CI:1.30-11.95), 2.32 times more likely for grade 2 (95% CI:0.54-10.07) and 3.48 times more likely for grade 3 (95% CI:0.35-35.19). A normal liver was 3.44 times less likely to have lymphomatous infiltration (p=0.007, 95% CI: 1.39-8.47). The odds of having a positive diagnosis of lymphoma in the spleen in dogs were 76.63 times more likely with leopard-spotted parenchyma (p<0.001, 95% CI:9.98-588.40) and 29.67 times more likely with splenomegaly (p<0.001, 95% CI:6.68-131.84). The grade given by the observers as to the likelihood of lymphomatous infiltration in the spleen (p<0.001) was 1.50 times more likely to be correlated with a positive result for grade 1 (95% CI:0.47-4.84), 12.88 times more likely for grade 2 (95% CI:2.96-56.04) and 138.52 times more likely for grade 3 (95% CI:16.65-1152.58). A normal spleen was 10 times less likely (p=0.036, 95% CI:1.16-83.33) and finely heterogeneous parenchyma was 3.97 times less likely (p<0.001, 95% CI:1.82-8.62) to have splenic lymphomatous infiltration. The odds of a diagnosis of multicentric T-cell lymphoma were 3.83 times more likely with finely heterogeneous splenic parenchyma (p=0.003, 95%CI:1.58-9.27) and 2.78 times more likely with splenic nodules (p=0.025, 95% CI:1.14-6.81). Leopard-spotted splenic parenchyma was 23.26 times more likely (p<0.001, 95% CI:5.13-111.11), splenomegaly was 4.37 times more likely (p=0.004, 95% CI:1.59-11.90) and hepatic nodules were 3.55 times more likely (p=0.058, 95% CI:0.96-13.16) to be associated with multicentric B-cell lymphoma.

On multivariable analysis in dogs, a normal ultrasonographic appearance of the liver was 3.44 times more likely to be associated with no lymphomatous infiltration (p=0.007, 95% CI:1.39-8.47). Leopard-spotted splenic parenchyma was 43.29 times more likely (p<0.001, 95% CI:5.41-346.39) and splenomegaly was 14.47 times more likely (p=0.001, 95% CI:2.94-71.34) to be associated with lymphomatous infiltration. Leopard-spotted splenic parenchyma was also 23.25 times more likely (p<0.001, 95% CI:5.13-111.11) to be associated with multicentric B-cell lymphoma. No significant interaction terms were identified in the final multivariable models.

DISCUSSION

Thorough staging of animals that are diagnosed with lymphoma prior to commencing treatment can provide valuable information to determine the extent of the disease, provide an indication of prognosis and monitor response to treatment (Marconato 2011). Although there is some controversy regarding whether there is prognostic difference between Stage III and IV in the World Health Organisation classification system, there is the potential, with advancing technology and knowledge, for more accurate stage identification (Nerschbach *et al* 2016) and consideration should be given to re-evaluating the survival relevance of these stages. Regardless of prognostic difference at diagnosis, the identification of tumour infiltration during restaging is important for clinical decision making (for example, whether the patient is in complete clinical remission or not) in the treatment phase.

In some cases, it may not be possible to perform fine needle aspirates due to financial constraints, operator skill or patient factors such as thrombocytopenia (Bigge *et al.* 2001). Additionally, in cats, there is an overlap in the cytological appearance of certain types of lymphoma and benign changes (Bertal *et al.* 2018) and a biopsy may not always be deemed feasible. In these cases, the clinicians may have to rely on diagnostic imaging findings alone for the staging. Radiography has been shown to be insensitive at detecting liver and spleen involvement in dogs (Blackwood *et al.* 1997) and these organs can often have a normal appearance on computed tomography despite lymphomatous infiltration (Jones *et al.* 2017). Ultrasonography is nowadays widely available, radiation-free, reasonably cheap and non-invasive.

The ultrasonographic features recorded in the liver and spleen of both dogs and cats are similar to those previously described (Nyland 1984, Lamb *et al.* 1991, Voros *et al.* 1991, Crabtree *et al.* 2010, Warren-Smith *et al.* 2012, Bertal *et al.* 2018, Harel *et al.* 2020). The agreement between the reviewers as to the likelihood of lymphomatous infiltration was high, especially for the assessment of the liver. This indicates that the sensitivity, specificity, accuracy, positive predictive value (PPV) and negative predictive value (NPV) of US in the detection of lymphomatous infiltration of the liver and spleen are reproducible.

Compared to a previous study with a smaller population, US as a tool for detecting lymphomatous infiltration of the liver was found to be dramatically less sensitive (16.7% versus [v] 72.7%), had a lower PPV (62.5% v 77.4%) and NPV (55% v 76.3%) and a lower accuracy (55.9% v 76.8%) (Crabtree *et al.* 2010). The sensitivity was more comparable to an older study which also included cats in their population (16.7% v 21%) (Lamb *et al.* 1991). It was however found to have a higher specificity (91% v 80.6%) in our study (Crabtree *et al.* 2010). The accuracy of US in the detection of lymphomatous infiltration in the liver mirrors that of general hepatic disease (55.9% v 50%) (Voros *et al.* 1991). When combining these results with the finding that an ultrasonographically normal liver is associated with no lymphomatous infiltration, the recommendation continues to be to obtain samples from the liver whenever possible, especially if there are any abnormal ultrasonographic findings. Interestingly, when cats are looked at individually, the NPV (90.9%) and accuracy (84.0%) were both higher than on a previous study (NPV 76.3% and accuracy 76.8%) (Crabtree *et al.* 2010) and compared to our canine population (NPV 46.1% and accuracy 49.0%). On the other hand, the PPV for cats was lower compared to both this previous study (33.3% v 77.4%) (Crabtree *et al.* 2010) and our canine population (33.3% v 69.2%).

When comparing US in the detection of lymphomatous infiltration of the spleen to this previous study, our results showed it was more specific (93.9% v 23.3%), had a higher PPV (93.4% v 64.6%) and a higher accuracy (82.6% v 68.1%) (Crabtree *et al.* 2010). It was however less sensitive (73.1% v 100%) and had a lower NPV (74.7% v 100%) (Crabtree *et al.* 2010). In our study, all values regarding the accuracy of US for the detection of lymphomatous infiltration are higher for the spleen compared to the liver. It should be highlighted that in both our study and the aforementioned older study, none of the dogs with lymphomatous infiltration of the spleen had an ultrasonographically normal spleen (Crabtree *et al.* 2010). Based on our results, the sensitivity of US for lymphomatous infiltration of the spleen in cats was low (28.6%) but the specificity and PPV were 100%.

Our study found that a normal ultrasonographic appearance of the liver in dogs is significantly associated with absence of lymphomatous infiltration. In a previous study, 11/14 dogs and cats with lymphoma had no abnormalities on US (Lamb *et al.* 1991). A possible explanation for this discrepancy is that in the previous study the radiologists used lower frequency transducers (5 and/or a 7.5MHz) and an older US machine. Higher probe frequency has been shown to improve the detection of subtle patterns and lesions (Bertal *et al.* 2018).

Leopard-spotted splenic parenchyma, also commonly called moth-eaten, Swiss-cheese or honeycomb (Bertal *et al.* 2018, Harel *et al.* 2020), is associated with lymphomatous infiltration of the spleen in dogs. Results from our data reflect the findings from a previous study (Crabtree *et al.* 2010) that this ultrasonographic pattern has a PPV of 100% in the detection of lymphomatous infiltration of the spleen in dogs. This pattern had no false positives, also leading to a specificity of 100%.

Splenomegaly in dogs was associated with lymphomatous infiltration in our study. Along with lymphomatous infiltration (Lamb *et al.* 1991, Blackwood *et al.* 1997), splenomegaly can also result from benign conditions (such as congestion (O’Brien *et al.* 2004), hyperplasia and extramedullary haematopoiesis) and other neoplasia types (Nerschbach *et al.* 2016). Mild to moderate splenomegaly is often disregarded as a significant finding in sedated dogs, especially when acepromazine or thiopental are used (O’Brien *et al.* 2004). Acepromazine administration has also been shown to increase reflectivity and attenuation of the spleen on US and it has therefore been suggested that the combination of hypoechogenicity and splenomegaly following acepromazine may be more evocative of a pathological process than isolated splenomegaly (O’Brien *et al.* 2004). No dogs in our study received thiopental and only 20 dogs received acepromazine prior to US. Of these, 9 had their spleen classified as enlarged but not in association with hypoechogenicity. All of these 9 dogs with splenomegaly also had other splenic abnormalities and had confirmed lymphomatous infiltration of the spleen. All of the dogs that received acepromazine but did not have lymphomatous infiltration of the spleen did not have splenomegaly. The findings from our study suggests that if the dog already has a diagnosis of lymphoma, then splenomegaly is suggestive of lymphomatous infiltration.

In our study, a leopard-spotted parenchyma of the spleen in dogs was associated with the B-cell immunophenotype of multicentric lymphoma, of which 79.5% of these cases were classified as diffuse large B-cell lymphoma (the remaining cases were not specified). Based on the literature search, no ultrasonographic feature has previously been associated with a specific immunophenotype of lymphoma. Immunohistochemistry or flow cytometry may not always be possible due to financial constraints and this finding could help refine the prognosis, as B-cell lymphoma typically have a more favourable prognosis than T-cell lymphoma (Vail *et al.* 2013).

With the addition of recent studies investigating the use of contrast enhanced US examination, there is the potential to further improve the diagnostic utility of US. Specific enhancement patterns have been shown to be suggestive of malignancy (O’Brien *et al.* 2004, Kanemoto *et al.* 2009, Nakamura *et al.* 2010) and additional lesions may only be visible on contrast-enhanced US (Kanemoto *et al.* 2009).

Limitations of this study include that fine needle aspirates were used as our gold standard to diagnose lymphomatous infiltration of the liver and spleen. There are wide-ranging figures of the accuracy of fine needle aspiration (Liffman & Courtman 2017, Bertal *et al.* 2018) but one study suggests that malignancy was always detected (Bonfanti *et al.* 2004); logically however there must be a lower limit of detection. In addition, all uncertain cytology results were excluded. Another limitation included the retrospective nature and therefore review of still ultrasound images. At our institution, multiple images of the liver and spleen are routinely obtained including any focal lesions, the shape of the margins, the relationship to other organs to assess for changes in size and a comparison to other organs in the same image to assess for changes in echogenicity. Cases were excluded if it was not possible to accurately assess the liver and/or spleen for the different classification features, however it remains possible that the still images that were assessed were not fully representative in every case. Due to the retrospective nature, there were also different operators and the use of different ultrasound machines and probes. However, we believe this is representative of normal practice and is not detrimental to the study. Finally, both radiologists were trained at the same institution, which may have introduced bias in the interpretation of images and the interobserver agreement.

In conclusion, US is a useful tool in the staging of lymphoma in dogs and cats. Detection of a normal liver, leopard spotted splenic parenchyma and splenomegaly in the dog can be used to aid assessment of lymphomatous infiltration of these organs. The presence of leopard-spotted splenic parenchyma can be used to predict the immunophenotype of multicentric lymphoma in the dog.

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LEGENDS

Figure 1: Examples of classification of the US patterns in the liver. A: Finely heterogeneous parenchyma, B: Strongly heterogeneous parenchyma, C: Hepatic nodule, D: Target lesion

Figure 2: Examples of classification of the US patterns in the spleen. A: Finely heterogeneous parenchyma, B: Leopard spotted parenchyma (also referred to as moth-eaten, Swiss-cheese or honeycomb patterns), C: Splenic nodule, D: Target lesion

No conflicts of interest have been declared.