Table 1. Median GSH:GSSG values from plasma and RBC hemolysate samples of anemic and non-anemic, healthy dogs, further divided into those with hemolytic and non-hemolytic anemias. Values with matching superscript letters represent significant differences.

	Plasma GSH:GSSH		RBC GSH:GSSG	
	Median	Range	Median	Range
Healthy	2.80[a]	0.09-29.91	7.22[c,d]	0.51-29.7
Non-Hemolytic Anemia	1.29[b]	0.01-7.09	4.11[c]	2.23-26.37
Hemolytic Anemia	7.96[a,b]	0.42-15.68	2.39[d]	0.4-30.83

We hypothesized that anemic dogs would have increased ROS detected by flow cytometry, and decreased concentrations of glutathione and vitamin E when compared to controls. We further hypothesized that markers of oxidative stress would be different in dogs with hemolytic anemia when compared to dogs with non-hemolytic anemia. Dogs were recruited from the hospitalized population of the veterinary teaching hospital. Dogs were eligible for inclusion to the study if their hematocrit or packed cell volume were <30%. Once included, medical records were reviewed to determine the most likely cause for the anemia. Causes were classified as either non-hemolytic or hemolytic. A second population of healthy control dogs was recruited as well. Forty-eight anemic dogs were included in the study, with 11 diagnosed with immune-mediated hemolytic anemia, and 37 diagnosed with non-hemolytic causes for their anemia. 20 healthy dogs were included in the study. Data were non-parametrically distributed with summary descriptive statistics presented as median [range] and assessed with the Wilcoxon rank sum test. Median GSH:GSSG values from plasma and hemolysate samples of anemic and non-anemic, healthy dogs, further divided into those with hemolytic and non-hemolytic anemias. Healthy dogs were found to have significantly higher RBC GSH:GSSG when compared to anemic dogs of both causes (Table 1, $p < 0.0001$). Dogs with hemolytic anemia had lower GSH:GSSH compared to dogs with non-hemolytic anemia, but this difference did not reach significance ($p = 0.081$). Dogs with hemolytic anemia had significantly higher plasma GSH:GSSG when compared to dogs with non-hemolytic anemia and healthy dogs (both $p < 0.0001$). There was no difference in intracellular ROS or vitamin E between groups. This study demonstrated a significant depletion of glutathione in anemic states, especially in dogs with hemolytic anemia. While the use of flow cytometry to label intracellular ROS did not find differences between groups, this method still shows promise. Prospective investigation of oxidative stress in immune-mediated hemolytic anemia is warranted, with an evaluation of the effect of antioxidant supplementation on these parameters.

ABSTRACT HM11 Identification of five new feline erythrocyte antigens based on the presence of naturally occurring alloantibodies

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Since the discovery of the feline Mik antigen, several studies have described blood incompatibilities unrelated to the AB system. Based on the presence of naturally occurring alloantibodies (NOAb), the purpose of this study was to begin mapping the corresponding feline erythrocyte antigens (FEA) behind these incompatibilities. By groups of six, 274 transfusion-naïve cats were prospectively evaluated for the presence of alloantibodies using a gel column crossmatch test. When non-AB-related alloantibodies were detected in a cat, its plasma was used to assess the presence or absence of the corresponding antigen in cats included thereafter in the study (blood typing). Plasma from 18 of 263 type-A cats (6.8%) caused 49 incompatible results out of 1257 crossmatches performed (3.9%). Presence of NOAb was statistically associated with an age of <2 years ($p = 0.04$). Using 7 of the 18 alloantibodies, systematic blood typing was performed, and results compared. Based on agreement analysis, 3 NOAb appeared to correspond to the same antigen (k^3 0.64); hence the identification of 5 different putative FEA (numbered in order of identification). FEA 1, 4, and 5 were most frequent with a prevalence of 84%, 65%, and 96%, respectively. Only FEA 1 was significantly associated with NOAb ($p = 0.005$), which were observed in 8 of 43 FEA 1-negative cats (19%). This study represents a first step of FEA's identification outside the AB system. Because of its prevalence and association with NOAb, FEA 1 may correspond to the lost Mik antigen. Banked alloantibodies will facilitate future studies, notably regarding their clinical relevance.

ABSTRACT HM12 Comparison of dogs treated for primary immune-mediated hemolytic anemia in Tuscany, Italy and Texas, USA

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Large-scale studies are needed to determine optimal treatment for canine immune-mediated hemolytic anemia (IMHA). Multicenter studies reduce the time for case enrollment, but differences in disease severity and outcome could complicate their interpretation. This retrospective study compared clinical characteristics between dogs treated for IMHA by veterinary teaching hospitals in Tuscany, Italy and Texas, USA between 2010 and 2018. As the two institutions used different diagnostic criteria, Tuscany cases (n = 48) were included if flow cytometry was positive for anti-erythrocyte antibodies and/or marked spherocytosis was reported. Texas cases (n = 43) were included if there was clinical evidence of hemolysis and marked spherocytosis and/or a positive saline agglutination test. Cases were excluded if a secondary IMHA was evidenced. Categorical data was compared by Chi-squared test and continuous data by Mann-Whitney test. The two populations did not differ significantly in age, but Texas dogs were more commonly neutered and had lower bodyweight. Chihuahuas and mixed breed dogs were most common in Texas and mixed breed dogs and Cocker Spaniels in Tuscany. Median hematocrit, erythrocyte regeneration response, occurrence of spherocytes and hyperbilirubinemia were not significantly different. Hemolyzed plasma and bilirubinuria were more common in Texan cases. CHAOS scores were not significantly different between groups. Texan cases

were more likely to be hospitalized at initial presentation, but there was no difference in survival to discharge between the two locations. Dogs treated for primary IMHA in a European or North American location were broadly similar, suggesting cases from both locations could be combined in future clinical trials.

ABSTRACT HM13 Chronic low-dose rapamycin does not cause red blood cell microcytosis in middle-aged, large breed dogs

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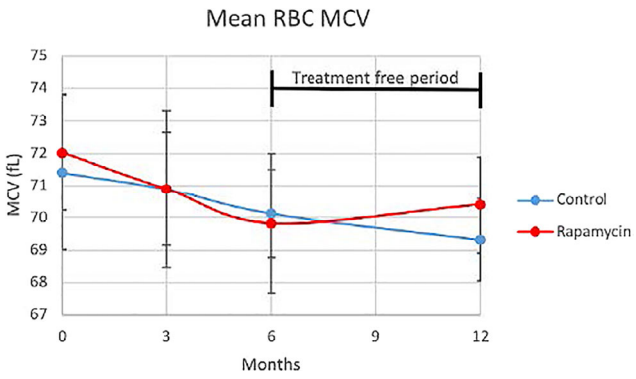
Rapamycin is a macrolide antibiotic that inhibits the mammalian target of rapamycin (mTOR), a protein complex involved in multiple metabolic pathways. Due to its ubiquitous nature, disruption of the mTOR complex via rapamycin has numerous systemic effects, including immunosuppression, anti-proliferative effects, and extension of lifespan in laboratory models. However, side effects, such as dyslipidemias and red blood cell microcytosis have been reported in humans and mice. This study aimed to determine the prevalence of progressive red blood cell microcytosis in middle-aged, large breed dogs who received long-term, low-dose oral rapamycin. Seventeen dogs (10 spayed females, 1 intact female, and 6 castrated males) were randomly assigned to receive rapamycin (0.025 mg/kg) or a placebo orally every Monday, Wednesday, and Friday for 6 months. All enrolled dogs underwent complete evaluations (physical exam, chemistry, CBC, urinalysis, and echocardiogram) at baseline, 3 months, 6 months, and 12 months (i.e., 6 months after discontinuation of treatment), and erythrocyte mean corpuscular volume (MCV) was recorded for each visit. There was no significant difference in MCV between the control and rapamycin treated groups at baseline (71.4 fL vs. 72.0 fL, respectively; p = 0.56), 3 (70.9 fL vs. 70.9 fL; p = 0.99), 6 (70.1 fL vs. 69.8 fL; p = 0.74), or 12 months (69.3 fL vs. 70.4 fL, p = 0.14). Additionally, there were no significant differences in the change in MCV between groups over the 6 month period (−1.3 fL [control] vs. −2.2 fL [rapamycin]; p = 0.18). In the cohort receiving rapamycin, there was a significant reduction in mean MCV from baseline to 6 months (72.0 fL vs. 69.8 fL; p = 0.03), but there was no significant difference in MCV between 6 and 12 months (69.8 fL vs. 70.4 fL; p = 0.54). In the control group, a significant decrease in MCV was observed from baseline to 12 months (71.4 fL vs. 69.3 fL; p = 0.04), a finding that reflects the need for more study subjects in order to better assess the potential of rapamycin-induced changes in MCV in healthy dogs.



ABSTRACT HP01 Evaluation of coagulation in dogs with gallbladder mucoceles

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Gallbladder mucoceles (GBM) are a common biliary disorder in dogs and are associated with inflammatory and thrombotic complications. Limited information is available on their coagulation status. The aim of this study was to assess coagulation in dogs with GBM. Twenty-three dogs with GBM identified on ultrasound were prospectively enrolled. Blood was collected at the time of GBM identification for determination of complete blood count, biochemical panel, packed cell volume, prothrombin time (PT), activated partial thromboplastin time (aPTT), factor VIII activity, fibrinogen, D-dimers, thromboelastrography (TEG), protein C activity (PC), antithrombin activity (AT), and von Willebrand's factor activity (vWF). Overall, when compared to the hospital generated reference range population, dogs with GBM had significantly decreased K values and increased angle, MA and G (p < 0.001). Based on G value, 19/23 dogs (82.6%) were classified as hypercoagulable and 4/23 (17.4%) were classified as normocoagulable. Four of 23 (17.3%) of dogs were classified as

Treatment	0mo MCV (fL)	3mo MCV	6mo MCV	12mo MCV	Treatment	0mo MCV	3mo MCV	6mo MCV	12mo MCV
Control	72.1	72	71	67.9	Rapamycin	72.9	73	72.4	72
Control	73.5	70.7	70.8	70.2	Rapamycin	75.7	72.9	73.1	72.7
Control	74.8	74.1	72.8	71.5	Rapamycin	69.3	68	67.9	68.5
Control	70.8	69.5	69.3	68.8	Rapamycin	71.7	67.6	69.5	69.1
Control	67	68.6	68.5	67.8	Rapamycin	73.3	72	71.6	70.4
Control	69.5	70.3	69.4	70.1	Rapamycin	71.5	68.4	66.5	69
Control	71.8	72.1	69.3	69.5	Rapamycin	71.7	72.9	68.6	70.5
Control	71.8	69.9	69.9	68.7	Rapamycin	70.8	73.4	69.5	71
Mean	71.4	70.9	70.1	69.3	Rapamycin	71.3	69.8	69.3	N/A
					Mean	72	70.9	69.8	70.4