Checklist:

The checklist is designed to assist applicants in the grant writing process. All items in the checklist are required to be completed prior to grant submission. Please include this completed checklist in your ACVO-VAF grant application. The grant application **must** include the following:

- ☒ Completed and signed cover page (page 1)
- ☒ Completed abstract and project duration (page 3)
- ☒ Complete description of the resources available for the proposed research (page 4)
- ☒ Complete research plan (beginning page 5; sections a through e)
- ☒ Complete literature cited (section f)
- ☒ Completed budget that does not exceed $5000 in total costs
- ☒ Complete description of investigators and key personnel
- ☒ Check if letters of cooperation are included
- ☒ Curriculum Vitae (use the 2-page NIH format)
- ☒ Check if appendices are included
- ☒ Check if IACUC or Hospital Executive Committee approval is included
- ☒ Check if an Informed Consent form is included
- ☒ All page restrictions are met
- ☒ PDF is one document and size restriction (no larger than 5MB) is met
- ☒ Submitted an electronic PDF to chandler.111@osu.edu

Scientific Abstract:

PURPOSE:
Sudden acquired retinal degeneration syndrome (SARDS) is a poorly understood disease causing acute blindness and signs of endocrinopathy in dogs. Circulating retinal autoantibodies have been found in some SARDS patients, similar to human autoimmune retinopathy. A viable treatment option needs to be established in patients affected with this disease. Our study serves to investigate the therapeutic effect of the immunomodulatory medication mycophenolate mofetil in patients affected with SARDS.

METHODS:
11 dogs diagnosed with SARDS in the acute stage will be administered mycophenolate mofetil (10mg/kg by mouth twice daily) for 6 weeks. Patient response will be evaluated pre- and post-treatment by complete ophthalmic examination, light and dark-adapted electroretinography, optical coherence tomography and retinal imaging, as well as serum biochemistry and autoantibody evaluation pre- and post-treatment. A client questionnaire and the canine visual function instrument will be utilized to evaluate vision based on owner perception. Patients will be recruited from a larger VAF-funded SARDS project that aims to define the role of autoimmunity in SARDS.

EXPECTED OUTCOMES:
We anticipate that mycophenolate mofetil will decrease autoantibody levels, improve clinical signs, serum biochemical alterations associated with endocrinopathy, and vision in treated animals.

SIGNIFICANCE:
SARDS is a devastating disease for pets (and owners) that are affected. SARDS not only leads to sudden blindness, but can also impact the endocrine system affecting quality of life for pet and owner alike. This will serve as a pilot study to determine whether mycophenolate mofetil is a safe and effective treatment for SARDS. The results of this study will be used to develop a double-blinded, placebo-controlled study of this therapy to prove treatment efficacy. Future directions also include determining an early-stage biomarker for SARDS, allowing for earlier and more successful therapeutic intervention.

Project Duration:

16-18 months
Patients are currently being recruited into the larger SARDS project. With owner consent, client-owned animals will be entered into this arm of the study. Diagnostics will be performed in part by the resident as patients are entered into the study. The resident's off-clinic time (totaling 4 weeks until July 2015 and then 8 weeks in the following 6 months) will be used for further data collection and analysis and autoantibody immunofluorescence and flow cytometry. Dr. Mowat and the retinal research lab technician will be assisting with the proposed research.

Resources:

Facilities:

Laboratory

The Ophthalmology research laboratory is housed in a recently refurbished multi-investigator laboratory in the main building of the NCSU College of Veterinary Medicine. Within this laboratory there are a number of facilities including (but not limited to):

- Adequate bench space to perform the proposed research
- Basic laboratory equipment (glassware, racks, IHC incubation chambers, pipettes and tips)
- Eppendorf centrifuge
- Refrigerator and -20°C freezer for sample storage
- -80°C Freezer for sample storage
- Flow cytometer core facility

Major laboratory equipment is detailed below

Personnel in this laboratory (including a dedicated ophthalmology research technician) have extensive expertise in using the equipment necessary to perform this study, and will be able to assist in training of study personnel in the safe and appropriate use of this equipment.

Clinical**:**

The NC State Veterinary Hospital is an integrated health care center for companion animals opened in 2011. The ophthalmology service has 4 dedicated exam rooms, a procedure room, a shielded electroretinography room, an ocular imaging room, and a kennel room for housing day patients.

Clinical equipment available:

- Slit lamp biomicroscope (Kowa SL-15 and SL-17)
- Tonometer (rebound TonoVet and applanation TonoPen Avia)
- Indirect Ophthalmoscope headset (Heine) and lenses (Volk)
- Direct Ophthalmoscope (Welch-Allyn)

Animals**:**

All participants will be recruited from client-owned animals presenting the NCSU Veterinary Teaching Hospital for evaluation of sudden blindness and subsequently diagnosed with SARDS. All clients must sign an informed consent prior to their pet's participation in the study. Enrollees will be housed in the ophthalmology kennel facilities during the day, if necessary, to complete all diagnostics.

Other**:**

We will consult with a paid statistician regarding data analysis prior to presentation/publication.

Institutional and IACUC approval has been granted for this study (available in appendices)

Major Equipment:

The following major clinical items are available in the Ophthalmology service in the Veterinary Teaching Hospital at NCSU:

Retinal imaging: Retcam II (Clarity Medical Systems)

Optical Coherence Tomography: Envisu R2000 (Bioptigen)

Electroretinography: Retiport 32 (Acrivet)

Research Plan:

A. Hypothesis and Specific Aims:

SARDS is a devastating disease resulting in destruction of the photoreceptor layer and irreversible blindness in affected animals. To date, there has been little discovered about the underlying pathophysiology of SARDS and no known treatments have been found. In recent studies, the role of autoimmunity in SARDS has come into question. Therapeutic intervention has been centered on the use of steroids, and has been largely unsuccessful may exacerbate clinical signs experienced by patients. Treatment directed at decreasing the autoimmune response, may alleviate clinical signs in patients with SARDS, improve quality of life, and vision.

Hypothesis: Mycophenolate mofetil will improve clinical signs, serum biochemical alterations associated with endocrinopathy, improve vision in treated animals, and will decrease the levels of circulating autoantibodies and T-cells,

Specific Aim #1: Determine the clinical effect of mycophenolate mofetil on vision and endocrinopathy in canine SARDS.

Specific Aim #2: Determine the effect of mycophenolate mofetil on the presence of circulating autoantibodies and T cell subsets in the bloodstream of SARDS patients.

B. Background and Significance:

Sudden acquired retinal degeneration (SARDS) is a devastating disease characterized by the acute onset of complete blindness. SARDS has no identified cause, and affected eyes appear normal when assessed with routine ophthalmoscopy. Diagnosis is based on history, clinical signs, normal appearing fundus with severely reduced electroretinogram (ERG) a- and b-wave amplitudes.[1-3] Median age of affected dog is 8.5 years with spayed females comprising roughly 60% of cases.[1] Dachshunds, Miniature Schnauzers, and mixed breed dogs have been over represented.[4] Animals may also be affected with systemic signs as well, including polydipsia, polyuria, polyphagia, and weight gain in up to 40% of cases.[1,5] Serum biochemical analysis may reveal abnormalities that mirror hyperadrenocorticism, though, with further investigation, most patients will not test positive for this disease.[5,6] Both blindness and clinical signs associated with SARDS have been implicated as cause for decreased quality of life, although the majority of owners with affected dogs would not recommend euthanasia following diagnosis.[7] Electroretinography remains the gold standard by which to confirm a diagnosis of SARDS since patients lack photoreceptor activity, though client cost may deter some pet owners from completing full diagnostics.[8] Optical coherence tomography (OCT) of SARDS-affected retinas shows progression to wide-spread retinal thinning affecting all cell layers in the later stages of disease.[8]

In humans, autoimmune retinopathy (AIR) shares many similarities to canine SARDS, as a form of rapid onset, bilateral blindness associated with the presence of retinal autoantibodies.[9] Although autoimmune retinopathy associated with cancer has been detected in several types of malignancies in people,[9,10] cancer has not been associated with the development of SARDS in dogs. [1] Human patients affect by AIR commonly have autoantibodies to retinal proteins.[9] Non

paraneoplastic AIR in people is variably responsive to treatments including high-dose steroids, plasmapheresis, and IV Ig.[9]

While SARDS is a commonly reported disease resulting in permanent blindness, the underlying pathogenesis and successful treatments to reverse blindness have not been fully defined.[7] The most convincing hypothesis is that of an autoimmune etiology, similar to human AIR. [3] Historical studies have shown minimal histological evidence of cell-mediated inflammation,[2,12] although subretinal macrophages were present early in disease in one study.[2] No studies have examined the types of circulating lymphocytes in SARDS patients. Two studies [11,13] failed to detect a difference in the presence of antiretinal antibodies between SARDS cases and normal controls. However, one study found that 25% of SARDS cases had anti-neuron specific enolase antibodies compared with 0% of control animals,[4] and another study found a higher proportion of SARDS cases had antiretinal antibodies as detected by western blot, ELISA and complement fixation, compared with normal dogs, dogs with uveodermatologic syndrome and dogs with progressive retinal atrophy.[14]

There is limited peer-reviewed information regarding successful treatment outcomes for SARDS.[3] Systemic high-dose steroids have been the historical mainstay of treatment (if any is instituted) for SARDS, with no published evidence of a positive effect.[7] In many cases, steroids are relatively contraindicated as a choice of immunosuppressant as at therapeutic doses, they may exacerbate the pre-existing endocrine symptoms including polyuria, polydipsia and polyphagia. Intravenous or intravitreal human immunoglobulin has been reported as a successful treatment for canine SARDS,[8] although no peer reviewed literature supports this theory. In human medicine, anaphylactic reaction to human immunoglobulin has been reported when delivered intravenously.[15]

Therefore there is a significant need to rigorously evaluate a safe, effective immunosuppressive treatment to treat SARDS in dogs.

Based on clinical availability, two drugs could possibly be selected for further clinical trials in SARDS patients: Cyclosporine and mycophenolate mofetil.

Cyclosporine is a commercially available non-steroidal immunomodulator that has been extensively used in human transplant patients.[16] It has been utilized in veterinary medicine for many diseases including immune-mediated hemolytic anemia, immune-mediated thrombocytopenia, and uveodermatologic syndrome. Many forms of cyclosporine are available but have unfortunately shown variable absorbency rates and efficacy between individuals. Cyclosporine can take 2-3 weeks to reach steady state concentration in the blood stream,[17] which may limit its effectiveness in SARDS, where morphologic changes in retinal structure is observed by 3 weeks.[2] In larger breed dogs, cyclosporine may be cost prohibitive and laboratory tests to determine serum levels in the individual are expensive. Trough levels are used to measure target blood concentrations but may not accurately reflect clinical response.[18] Considering these limitations, cyclosporine might not reflect a first-line, sole treatment option for SARDS patients.

Mycophenolate mofetil has been used as a sole agent to treat immune-mediated diseases in veterinary patients as a safe and effective alternative to other drugs.[18] The active form of mycophenolate targets T and B-lymphocytes preventing differentiation, proliferation, and the formation of immunoglobulin. Mycophenolate has a relatively short onset of action (2-4 hours) and

is tolerated well in most animals.[18] Side effects such as diarrhea can be noted in up to 20% of cases,[19] but are commonly relatively mild and can typically be alleviated with a decrease in dosage. Although cost may still be significant for some owners, it is less expensive than cyclosporine and may have a more predictable outcome across patients, while side effects are relatively mild and easy to control.

Our study therefore proposes to study mycophenolate mofetil as a potential sole-agent for the treatment of SARDS in canine patients. We will use clinical cases already recruited to our recently funded SARDS study and study the clinical, biochemical and immune effects of treatment with mycophenolate mofetil. Should the results of this initial study be positive, we would plan to seek funding for a larger masked clinical trial to definitively prove efficacy.

C. Preliminary Data:

We have examined our clinical caseload over the past 5 years to define patient population and predict case enrollment. A total of 81 SARDS cases were seen over the 5-year period, with 47 cases confirmed at NCSU by ERG. The median number of cases examined at NCSU for a first appointment referral for SARDS for the last 5 years is 15. Based on these data, we anticipate recruitment of our planned 11 cases to take approximately 9-12 months.

D. Experimental Plan:

Specific Aim #1: Determine the clinical effect of mycophenolate mofetil on vision and endocrinopathy in canine SARDS.

Rationale:

The goal is to evaluate the clinical effect of a novel therapeutic for the treatment of SARDS. The effect of mycophenolate mofetil on clinical signs, serum biochemical alterations associated with endocrinopathy, and vision in SARDS patients will be evaluated after 6 weeks of treatment.

Experimental Design:

11 participants will be recruited from NCSU veterinary ophthalmology caseload. New patients presenting for sudden blindness that have signs consistent with a diagnosis of SARDS will be eligible for inclusion. All patients will be already enrolled into our funded SARDS study (with owner consent), which has full institutional approval. Per the study design, animals will undergo complete ophthalmic exam, as well as ERG (Acrivet), OCT (Bioptigen), retinal imaging (Retcam II, Clarity Medical Systems), with complete blood count, serum biochemistry, urinalysis and ACTH stimulation testing at the start of the study. Clients who wish to participate in the treatment study will sign a further consent form (consent form is included in the appendix). Patients will be treated with 10mg/kg of mycophenolate mofetil orally twice daily for a period of six weeks. Costs for medications will be covered by Stokes Pharmacy (see letter of collaboration). At the end of the treatment period, patients will be reassessed with same diagnostic tests performed.

Client questionnaire will be included to assess perceived visual function of each patient (included in appendix). The canine visual function instrument (CVFI) as presented by Dr. Bill Miller at the ACVO conference 2015 [20] will be used. Briefly, the CVFI will assess visual function based on behavior and vision quality using specific questions on owner survey. CVFI was recently presented at the ACVO conference in Coeur d'Alene and found a positive correlation between owners' perception of vision and clinical diagnosis.[16]

Power analysis: Mycophenolate mofetil has been used as a sole agent to treat immune-mediated thrombocytopenia with a 100% remission rate.[21] We will be obtaining paired samples pre- and post-treatment, therefore will compare parameters using a paired t-test. Reviewing our 81 historical cases at NCSU for the last 5 years, the mean ± standard deviation for ERG b-wave amplitude in SARDS patients is 14 ± 13 μV. To detect a 50% improvement (to a mean of 28μV) as has been shown in human trials of immunosuppressives for AIR 5 we would need to recruit 9 patients (biomath.info power analysis program). If we anticipate a 20% dropout rate due to adverse effects of mycophenolate, the recruitment number would be 11. We do not anticipate recruitment to take longer than 1 year based on our historic caseload for SARDS at NCSU and recruitment efforts associated with the funded study.

Data Analysis:
Pre- and post- treatment diagnostic tests will be compared for each patient, including changes in serum biochemical parameters, pre-and post- ACTH cortisol, complete blood count, and urinalysis parameters. We will compare ERG a- and b-wave implicit times and amplitudes (dark and light-adapted values and cone flicker values) pre and post-treatment. We will compare total retinal thickness and the thickness of individual retinal layers in the area centralis, visual streak and superior and inferior retinal areas pre- and post-treatment. A paired student's t-test will be used to compare pre and post-treatment values, with significance set at $P<0.05$. The canine visual instrument and client questionnaire will be used to compare pre-and post treatment client assessment of vision and quality of life.

Expected Results:
We anticipate that mycophenolate mofetil will improve clinical signs, serum biochemical alterations associated with endocrinopathy, and improve vision in treated animals.

Limitations, Potential Pitfalls, and Alternative Approaches:
The study assumes an immune-mediated pathogenesis, which is not completely proven in SARDS, although previous studies support this hypothesis. Dr. Mowat's larger grant will examine the basis of autoimmunity in SARDS. It is possible that the drug will be ineffective, or have limited effect. We may need to increase the dose or add supplementary medications, which would form part of a future study.

Specific Aim #2: Determine the effect of mycophenolate mofetil on the presence of circulating autoantibodies and T cell subsets in the bloodstream of SARDS patients.

Rationale:
We wish to examine whether treatment with mycophenolate mofetil reduces the presence of circulating autoantibodies to canine retinal, neuronal and adrenal tissue, and levels of circulating B-cells and T-cell subclasses.

Experimental Design:
Baseline autoantibody detection for SARDS patients and appropriate controls forms part of the funded ACVO VAF SARDS grant awarded in 2015 to Dr. Mowat. This proposal covers a comparative assessment of the presence of autoantibodies following treatment with the immunomodulatory drug mycophenolate mofetil, Such a study assessing the effect on autoantibody production has not been performed in any SARDS therapeutic trial reported to-date.

Dr. Mowat's funded study will archive peripheral blood mononuclear cells from patients pre-treatment. We therefore also plan to assess the effect of treatment on T cell and B cell numbers using flow cytometric analysis.

Direct immunofluorescence protocol
Serum from all animals will be individually incubated with canine tissues (collected from cadavers for the funded ACVO VAF SARDS grant). We will perform no serum control incubations concurrently with all patient sample incubations to control for non-specific binding to tissue by secondary antibody. We will also utilize primary conjugated fluorophore to minimize non-specific binding by secondary antibodies. We will follow protocols described for canine specific IgG,[22] IgA, IgE and IgM [23] antibodies as previously described by collaborators at NCSU. A nuclear counterstain will be utilized to identify retinal cell layers appropriately.

Imaging
We will utilize a fluorescence microscope to collect images of our tissue sections for analysis. Stained and unstained sections will be archived at -80°C to allow further analysis to be performed as needed. Images of immunohistochemistry will be collected for scientific publication. Although some researchers advocate quantifying immunofluorescence intensity to determine the extent of autoantibody production, it is the opinion of the authors that this is not sufficiently robust to allow statistical analysis, due to minute differences in protocol (e.g. secondary antibody concentration, duration of incubation, temperature) potentially influencing the intensity of staining independent of the amount of autoantibody present in patient serum. We will grade the images according to the cellular layer stained (e.g. outer nuclear layer, outer segments etc.) and the presence or absence of staining and will present summary statistics on those data to generate a picture of the extent of autoantibody production to different tissues between pre- and post-treatment samples.

Flow cytometry
Peripheral blood mononuclear cells will be harvested from whole blood using a commercially available blood tube (BD CPT tube, VWR). Cells will be frozen at -80C or liquid nitrogen until assay. NCSU CVM has a core facility running flow cytometric analysis for researchers and clinicians. We will use previously optimized antibodies for canine CD4/CD8+ T-cells (Invitrogen) and B-cells (CD79a; Novus Biologicals). This will allow us to assess the total numbers of T-cells and B-cells and the ratio between CD4 and CD8+ T-cells. The findings of this arm of the study might lead to future studies such as T-cell cytokine release assays to further define the role of different lymphocytes in SARDS pathology.

Data Analysis:
Slides labeled and imaged for autoantibody presence will be assessed in a masked fashion to limit investigator bias. Pre- and post-treatment autoantibody scores will be compared using descriptive statistics and a paired student's t-test. Total numbers of T and B-cells and the ratio of CD4 to CD8+ T cells will also be compared using a paired t-test.

Expected Results:
We anticipate that treatment with mycophenolate mofetil will reduce the levels of circulating autoantibodies to multiple organs in SARDS patients. There will be a reduction in both T and B cell numbers and the ratio of CD4 to CD8 T-cells will increase, reflecting a greater number of T helper cells compared with cytotoxic T cells.

Limitations, Potential Pitfalls, and Alternative Approaches:

The underlying autoimmune nature of SARDS is still in question, therefore it is possible that mycophenolate will have minimal clinical effect on the disease, having no effect on levels of circulating autoantibody production despite suppressing lymphocyte numbers. However, the publishing of definitive evidence of a lack of an effect of an immunosuppressive medication would add significantly to the paucity of literature addressing this important question.

E. Time Line for the Experimental Plan:

Patient recruitment will begin at the time of study funding. The larger funded SARDS grant (examining clinical parameters and presence of circulating autoantibodies) is currently enrolling patients.

Specific aim 1:

Patient recruitment (including 6 weeks of treatment): 12-14 months
Data analysis: 2-3 months

Specific aim 2:

Patient recruitment (including 6 weeks of treatment): 12-14 months
Assay performance: 2-3 months
Data analysis: 2-3 months

F. Literature Cited:

1. Carter RT, Oliver JW, Stepien RL, et al. Elevations in sex hormones in dogs with sudden acquired retinal degeneration syndrome (SARDS). *Journal of the American Animal Hospital Association.* 2009; 45: 207–214.
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13. Keller RL, Kania SA, Hendrix DV, Ward DA, Abrams K. Evaluation of canine serum for the presence of antiretinal autoantibodies in sudden acquired retinal degeneration syndrome. *Veterinary Ophthalmology*. 2006; 9: 195-200.
14. Bellhorn RW, Murphy CJ, Thirkill CE. Anti-retinal immunoglobulins in canine ocular diseases. *Seminars in Veterinary Medicine and Surgery (Small Anim)*. 1988; 3: 28-32.
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16. Laupacis A, Keown PA, Ulan RA, Mackenzie N, Stiller CR. Cyclosporin A: a powerful immunosuppressant. Canadian Medical Association Journal. 1982; 9: 1041-1046.
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Estimated Budget: Not to exceed 2 pages

Animals, Per Diem, and/or Owner Compensation:
Not Applicable

Equipment:
Not Applicable

Expendable Supplies:
These supplies will facilitate the performance of the ERG component of the clinical trial, the autoantibody assessment and flow cytometry, an estimated patient weight of 15kg was used for calculations. 11 patients are factored into the calculations. Stokes Pharmacy will pay for mycophenolate.

Disposables for ERG (electrodes)……..……..$150
Tropicamide (1) ……...$14
Proparacaine (1)……..$42
Goniovisc (1) ….……..$45
Sedation drugs @ approx. $6/patient ….……..$66
IV catheters, syringes and blood collection tubes @ approx. $15/patient……..$165
Disposables for serum autoantibody analysis (slides, coverslips, mounting media)……....$100
Flow cytometer usage fees (2 hours)…….……..$300
Flow cytometry disposables (tubes, diluent)……...$100
Flow cytometry antibodies (CD4/8 T cells Invitrogen, CD79a B cells Novus)……...$819
Total…..$1801

Other:
A discount on clinical tests (approved by the hospital board) is factored in for all line totals. 11 patients are factored into the calculations.

Clinical ERG/OCT/imaging @ $100 ea……...$1100
Complete blood count @ $36 ea……..…$396
Serum biochemistry @ $51 ea……..……$561
Urinalysis @ $27 ea……..……...$297
Serum cortisol @ $42 ea (pre and post)……..……$924
ACTH for stimulation testing @ approx. $25/patient…….……....$275
Total…..$3553

Total Direct Project Costs $5354
The remainder of the costs will be funded using Dr. Mowat's NCSU faculty startup funds
The ACVO-VAF does not support any institutional F&A $0

Total Costs Requested from ACVO-VAF Resident Research Fund…….$5000

Investigator Information:

A. Role of Investigators and Key Personnel:

Dr. Whitney Young (60%): Dr. Young is a resident in comparative ophthalmology, interested in retinal disease. She spent 1 year as a post-doctoral fellow in a neuro-retina research laboratory and regularly performed and interpreted ERG, OCT, and retinal images on multiple canine colonies. She has performed hundreds of eye exams.
Dr. Young will assist in case recruitment, pre- and post-treatment clinical evaluation, blood collection and will perform the assays for autoantibodies and T cell cytokine secretion.

Dr. Freya Mowat (10%): Dr. Mowat is a diplomate of both the American and European Colleges of Veterinary Ophthalmologists and studied retinal electrophysiology as part of her PhD. She was recently awarded a national foundation grant to study SARDS. She has extensive experience with the aforementioned modalities and has performed thousands of eye exams.
Dr. Mowat will assist in case recruitment, pre- and post-treatment clinical evaluation, and will assist in the design and implementation of the assays for autoantibody detection and T-cell cytokine secretion

Jon Hash (25%): Jon is a certified veterinary technician with a full-time position in Dr. Mowat's laboratory.
Jon will assist in case recruitment, pre- and post-treatment clinical evaluation, blood collection and will provide technical assistance for laboratory assays.

Dr. Katherine Lunn (5%): Dr. Lunn is a diplomate of the American College of Veterinary Internal Medicine.
Dr. Lunn will provide guidance and assistance in the dosing of mycophenolate mofetil, and management of potential side effects if seen.

B. Letters of Cooperation:

1. Letter from Dr. Bill Miller describing the collaborative use of the Canine Visual Instrument developed for owner assessment of vision.
2. Letter from Samantha Newton describing Stokes pharmacy's contribution of mycophenolate mofetil for treatment of animals enrolled in this study.

C. Biosketch Forms:

1. Dr. Whitney Young
2. Dr. Freya Mowat
3. Dr. Katharine Lunn

Appendices:

1. IACUC approval form. We confirm that research will be conducted in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and will adhere fully to the ACVO-VAF's Research Animal Involvement Policy
2. Owner informed consent form.
3. Hospital board approval application form
4. Client questionnaire (in-house)