**Project Title:** Evaluation of the therapeutic efficacy and changes in cytokines in cerebrospinal fluid and plasma in dogs with canine distemper encephalitis treated with intrathecal live Newcastle Disease Virus vaccine.

**Primary Investigator:** Kenneth R. Harkin, DVM, DACVIM, Professor and Head, Section of Medicine

**Co-Investigator:** Melinda Wilkerson, DVM, PhD, DACVP, Professor and Director, Clinical Immunology/Flow Cytometry Laboratory

**Mailing Addresses:**
Kenneth R. Harkin, DVM, DACVIM
106 Mosier Hall
College of Veterinary Medicine
Manhattan, KS  66506-5606
harkin@vet.k-state.edu

Melinda Wilkerson, DVM, PhD, DACVP
K242 Mosier Hall
College of Veterinary Medicine
Manhattan, KS  66506-5606
wilkersn@vet.k-state.edu

**Amount Requested:** $35,833
Study Description and Justification

Canine distemper virus demyelinating leukoencephalomyelitis (CDV-DL) is a devastating and almost routinely fatal disease of dogs that have not been vaccinated, a common problem in dogs presented to shelters. Despite best intentions and practices of shelters, the ubiquitous and insidious nature of canine distemper virus (CDV) ultimately leads to the development of the various forms of canine distemper virus infection in a portion of dogs adopted from shelters. CDV has been responsible for large-scale shelter outbreaks resulting in the loss of life in numerous shelter dogs before they have had a chance for adoption. To date there are no approved therapies for CDV in any form.

An unorthodox approach to the treatment of CDV-DL was first introduced in the early 1970s by Dr. Allison Sears, DVM, now retired in Park City, UT. He introduced the concept of using the live virus vaccine for Newcastle Disease (NDV) (a paramyxovirus like CDV) to stimulate the dog’s immune system to clear the CDV. His protocols have evolved through the years and are promoted on the website www.kindheartsinaction.com. This experimental and unconventional therapy appears to offer a glimmer of hope for a specific therapy for CDV-DL, a disease that to this point carries a grave prognosis.

We believe that this unorthodox method of treatment of CDV-DL has merit and deserves more rigorous investigation given the results that we have witnessed in our small set of patients (see below). This therapy has the potential to not only reduce the mortality associated with CDV-DL, but allow affected dogs to recover much of their lost function, allowing them to live longer and fuller lives with their adoptive families.

Canine distemper virus paradoxically results in systemic immunosuppression while at the same time inducing a strong localized immune response within the brain leading to neuronal necrosis and severe demyelination. Previous studies identified high levels of specific inflammatory cytokines in the brain and CSF of dogs with CDV-DL.1 CDV-DL represents an animal model of Multiple Sclerosis (MS), a demyelinating disease in people.2 Similar to CDV-DL, the white matter destruction in MS is a consequence of an autoimmune attack against myelin antigens. Although the etiology of MS is unknown, the production of anti-viral antibodies intrathecally may initiate disease.3,4 Similarly, although the chronic demyelination of CDV-DL is immune-mediated, there is evidence that viral persistence and spread through the central nervous system is a necessary component of this demyelination.4 Identifying the changes that occur in cytokine expression is the first step in understanding whether intrathecal NDV inoculation can modify the immune response in CDV-DL. We hypothesize that pro-inflammatory cytokines will decrease and anti-inflammatory cytokines will increase in the CSF as a result of NDV vaccine therapy concurrent with the clearance of CDV from the CNS.

**Project Summary:**

**Background:** In May, 2012, a 1-year-old Border collie was presented to the Kansas State University-Veterinary Health Center (KSU-VHC) with a presumptive diagnosis of CDV-DL. This dog was blind, paralyzed, and had severe myoclonus at the time of presentation and we confirmed the diagnosis of CDV-DL. The client requested the intrathecal inoculation of live Newcastle Disease Virus (NDV) vaccine (B1 type, LaSota strain). Given that there were no other options, save euthanasia or natural death to the disease, the decision was made to proceed with the intrathecal injection of NDV vaccine. The patient was discharged two days later and the client kept us up to date with the dog’s progress. In September, 2012 the dog was re-evaluated at KSU-VHC. At this time the dog was visual and ambulatory with only residual myoclonus of the right rear leg. Diagnostic testing revealed the CSF was now negative for CDV by PCR and there was no longer an inflammatory pleocytosis. Additional follow-up via telephone and videos have shown resolution of the myoclonus and the ability of the dog to now herd sheep.

Five additional dogs have been treated, including three 10-12 week old mixed breed puppies with the acute systemic form, an 8-month-old Shepherd cross with the chronic nervous form two weeks after recovery from the catarrhal form, and a 4-month-old, Labrador retriever cross with the chronic nervous form two weeks after recovery from a mild episode of the acute catarrhal form. Of the three puppies, one died in-hospital from severe systemic involvement, whereas the other two were successfully discharged following the therapy. The 8-month-old dog was paralyzed and had frequent seizures at the time of admission. Six days post-treatment the dog was able to get to a sternal position and eat and drink on its own, however recovery plateaued there. The 4-month-old Labrador retriever cross had improved ambulation and was no longer blind at one week and has continued to improve one-month post-treatment (per owner and referring veterinarian).

**Patient enrollment:** Ten dogs will be enrolled. Confirmation of CDV-DL will be made with the combination of neurological examination and conjunctival scrape and whole blood polymerase chain reaction testing (PCR) for CDV. Inclusion will be at the discretion of the primary investigator (KRH) to ensure that the dog is a viable candidate for therapy with intrathecal NDV. At this stage, however, we will limit inclusion to dogs with severe neurological disease from CDV-DL (including but not limited to ataxia, blindness, myoclonus, hypermetria, and paralysis) that are consistent with demyelination and with a guarded to grave prognosis.

**Patient evaluation:** Routine diagnostics that will be performed on all patients will include complete blood count, serum biochemistry profile, urinalysis, fecal flotation, and CDV PCR on whole blood and conjunctival scrapes. Additional diagnostics that may be performed at the discretion of the clinician (KRH) to ensure the appropriate clinical management of each patient may include, but are not limited to, 4DX SNAP (heartworm, Lyme, Anaplasma, Ehrlichia), and abdominal or thoracic radiographs. The follow-up will be performed at 3-4 months. We do not anticipate that magnetic resonance imaging (MRI) of the brain will be required, but may be performed to rule-out concurrent disease.

**Patient management:** Pending the results of CDV PCR testing, patients will be either hospitalized in the Isolation ward and given appropriate, individualized medical care or sent home with the owner if no specific medical management is required.

**Intrathecal injection of live Newcastle Disease vaccine:** Dogs will be anesthetized by the anesthesiology service according to the protocol deemed safe for each individual patient by a board-
The fur will be clipped over the dorsal cervical region and aseptically scrubbed as is routine for a cerebrospinal tap at the atlanto-occipital (A-O) joint. A 21-gauge, 1.5 inch spinal needle will be inserted into the subarachnoid space at the A-O joint by a board-certified internist (KRH), cerebrospinal fluid (approximately 1.5-2 ml) will be removed for CSF analysis, cytokine analysis, CDV antibodies, and CDV PCR testing. With the needle still in place, 0.1 ml/10 kg (maximum of 0.5 ml) of live Newcastle Virus vaccine (B1 strain, LaSota strain, Zoetis Inc.) will be injected into the subarachnoid space, after which the needle will be withdrawn. The patient will then be recovered from anesthesia and carefully monitored per hospital protocol.

**Patient management post-injection:** We have not witnessed any complications in the six dogs in which we have performed this procedure. However, clinical signs consistent with meningitis have been reported at [www.kindheartsinaction.com](http://www.kindheartsinaction.com) from this procedure. We theorize that this has been the consequence of poor technique during the CSF tap and injection and not directly caused by the vaccine itself. However, if signs of meningitis were to develop in the post-injection period, these dogs would be humanely euthanized and a full necropsy performed. Once the dog has fully recovered from anesthesia with no apparent complications for 24 hours they will be released to the owner. Owners will be contacted weekly for the first month, then monthly to record the progress prior to the recheck at 3-4 months.

**Cytokine analysis:** A multiplex bead cytokine capture assay system (MagPix, Millipore) will be used to measure cytokines in the CSF and serum. This assay can detect multiple proteins (i.e. cytokines) in a single tube. We will use the MILLIPLEX MAP Canine Cytokine/Chemokine Magnetic Bead Panel (EMD Millipore) to assay inflammatory and anti-inflammatory cytokines including IFN-γ, IL-2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, IP-10, KC-like, MCP-1, TNF-α, and GM-CSF. In addition, IL-1 and IL-12, and TGF-beta will be added by customization or by using specific ELISA kits. Samples will be collected and frozen at -80°C until they can be analyzed together in duplicate.

**CSF Analysis for lymphocyte subset typing:** If a CSF pleocytosis is present, CSF lymphocytes will be immunophenotyped by flow cytometry for the distribution of activated T-cell and B-cell subtypes.

**Additional diagnostics performed at follow-up for the study:** Canine distemper PCR (CSF, whole blood and conjunctival scrape), Canine distemper antibodies (serum and CSF), CSF analysis, Newcastle Disease Virus PCR (CSF), complete blood count, and serum biochemistry profile.

**Expectations:** We anticipate a 60-80% clinical recovery rate, as is anecdotally reported and is consistent with our clinical experience. We anticipate a normalization of CSF cytokines with therapy.

**Method of Analysis:** The clinical response will be reported by a descriptive analysis in the absence of a control population. Routine statistical comparison of CSF and blood cytokine analysis will be performed.

**Estimated time of completion:** We anticipate that this project should be completed within 12 months, however as with any study involving clinical patients, it is possible that the study may need to be extended to 24 months.
## Proposed Itemized Budget: (anticipating standard annual price adjustment for KSU-VHC charges)

<table>
<thead>
<tr>
<th>Item Description</th>
<th>Cost</th>
</tr>
</thead>
<tbody>
<tr>
<td>MILLIPLEX MAP Canine Cytokine/Chemokine Magnetic Bead Panel:</td>
<td>$5,525</td>
</tr>
<tr>
<td>MILLIPLEX customization kits (TGF, IL-12, IL-1) +ELISA:</td>
<td>$3,250</td>
</tr>
<tr>
<td>MagPix calibration/fluidics and reagents</td>
<td>$ 300</td>
</tr>
<tr>
<td>Flow cytometry reagents/testing for lymphocyte class:</td>
<td>$2,000</td>
</tr>
<tr>
<td><strong>Patient Fees:</strong></td>
<td></td>
</tr>
<tr>
<td>Examination (110X10)</td>
<td>$1,100</td>
</tr>
<tr>
<td>CBC/Chemistry/UA/fecal flotation (120X10) initial</td>
<td>$1,200</td>
</tr>
<tr>
<td>CBC/Chemistry profile follow-up (60 X 10)</td>
<td>$  600</td>
</tr>
<tr>
<td>Additional diagnostics (4DX, radiographs,MRI) estimated</td>
<td>$1,800</td>
</tr>
<tr>
<td>Anesthesia (Fee + Venous Catheter): $100 X 10</td>
<td>$1,000</td>
</tr>
<tr>
<td>Anesthesia (Fee + VC) follow up: $100 X 10</td>
<td>$1,000</td>
</tr>
<tr>
<td>Newcastle Vaccine (40/vial X 10 vials)</td>
<td>$  400</td>
</tr>
<tr>
<td>Canine Distemper PCR (CSF, conjunctival,whole blood): $120 X 10</td>
<td>$1,200</td>
</tr>
<tr>
<td>Canine Distemper PCR follow-up: 120 X10</td>
<td>$1,200</td>
</tr>
<tr>
<td>CSF analysis (initial and follow-up) $50 X 20</td>
<td>$1,000</td>
</tr>
<tr>
<td>CSF tap (initial and follow-up) $80 X 20</td>
<td>$1,600</td>
</tr>
<tr>
<td>Newcastle PCR $80 X 10</td>
<td>$  800</td>
</tr>
<tr>
<td>CDV antibodies (CSF, serum) $60 X 20</td>
<td>$1,200</td>
</tr>
<tr>
<td>Recheck examination $20 X 10</td>
<td>$  200</td>
</tr>
<tr>
<td>Daily care (isolation) 120/day X 4 days X 10</td>
<td>$4,800</td>
</tr>
<tr>
<td>In-hospital medications (estimated) 200 X 10</td>
<td>$2,000</td>
</tr>
<tr>
<td>Daily care (follow-up) 40/day X 1 day X 10</td>
<td>$  400</td>
</tr>
<tr>
<td>10% Indirect costs (facilities and overhead)</td>
<td>$3,258</td>
</tr>
<tr>
<td><strong>Total:</strong></td>
<td>$35,833</td>
</tr>
</tbody>
</table>
KENNETH R. HARKIN, DVM, Diplomate ACVIM (Internal Medicine)

EDUCATION
Doctor of Veterinary Medicine: Iowa State University, Ames, Iowa. Degree conferred May 1989

WORK EXPERIENCE
Kansas State University, Department of Clinical Sciences, Veterinary Medical Teaching Hospital
1800 Denison Ave., Manhattan, KS 66506-5606
July 1997-March 1998: Clinical Instructor in Internal Medicine
April 1998-present: Assistant Professor in Internal Medicine (1998-2004); Associate Professor in Internal Medicine—Tenured July, 2004; Professor in Internal Medicine—Promoted July, 2010

SELECTED PUBLICATIONS


LECTURES (current)
Companion Animal Medicine, CS 709
Clinical neurology, 13 hours, 1998-present
Hematology, 5 hours, 2004-present

Companion Animal Medicine, CS 711
Gastroenterology and Hepatology, 15 hours, 2002-present

GRANTS


GRADUATE STUDENTS
Ram Raghavan. Doctoral thesis: Geospatial risk factor analysis for canine leptospirosis in the Great Plains. PhD awarded May, 2011. Co-major Professor (T.G. Nagaraja, Department of DMP, also served as co-major Professor).

AWARDS
Norden-Pfizer-Zoetis Distinguished Teacher of the Year, Kansas State University, 2001, 2008, 2013
Iman Outstanding Teacher Award, 2013
Novartis Teaching Award, 2012
Dr. William and Deanna Pritchard Veterinary Service and Outreach Award, 2012
Kansas Veterinary Medical Association KSU Distinguished Service, 2006
CURRICULUM VITAE

MELINDA J. WILKERSON

Education:
Washington State University, CVM 1989-1994 Ph.D.
University of Missouri, CVM 1985-1989 D.V.M., M.S.
St. John's School of Medical Technology 1981-1982 M.T.A.S.C.P.
Southwest Missouri State University 1980-1981 B.S.
Central Missouri State University 1977-1980

Professional Experience:
2012 Full Professor
1/2011 – present Coordinator of Clinical Pathology Residency Training Program
10/2010 - present Coordinator of Digital Information, Instruction, and Learning
2007 – 2010 Interim Associate Dean of Academic Programs, Kansas State University,
College of Veterinary Medicine
Oct. 1996-Present Associate Professor (2003), Assistant Professor (1996)
Director of Clinical Immunology/ Flow Cytometry Laboratory, Clinical
Pathology service, Department of Diagnostic Medicine/Pathobiology,
CVM, Kansas State University, Manhattan KS

Speciality Certification/Training:
Diplomate of the American College of Veterinary Pathology,
Certified Becton Dickinson FACScan Key Operator, (1997) San Jose, CA.

Awards
2008, Outstanding Woman Veterinarian of the Year Award, Association for Women Veterinarians
Foundation

Formal Course Instruction:
DMP 705 (3 credit hr), Principles of Veterinary Immunology, (Spring)
DMP 878 (2 credit hr), Theory and Applications of Flow Cytometry (Fall)
DMP 850 (3 credit hr), Domestic Animal Immunology (Fall)

GRADUATE STUDENT TRAINING (MOST RECENT):
- 2005: Kekha Kempegowda, completed MS. Thesis: Development and evaluation of a multiplex assay to measure bovine IgG1 and IgG2 using microspheres and flow cytometry

CLINICAL PATHOLOGY RESIDENT TRAINEES AT K-STATE

<table>
<thead>
<tr>
<th>Name</th>
<th>Training</th>
<th>ACVP board exam</th>
<th>Post training position</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adi Wasserkrug Naor</td>
<td>2013-2016</td>
<td>scheduled for 2016</td>
<td></td>
</tr>
</tbody>
</table>
Rachel Holicky  2013-2016  scheduled for 2016
Mandy Meindel  2011-2014  scheduled for 2014
Kate Pennick  2010-2013  passed 3 of 4 parts of ACVP exam
Don Petersen  2009-2011  ACVP Diplomate  Abaxis Labs
Casey Wood  2005-2008  ACVP Diplomate  IDEXX Labs
Merhdad Ameri  2004-2008  ACVP Diplomate  Amgen, Los Angelas, CA
Robert Di Terlizzi  2004-2007  ACVP Diplomate  Univ. Penn
Tanya Grondin  2004-2007  ACVP Diplomate  Practice
Balazs Szladovitis  2003-2006  ACVP Diplomate  Royal Veterinary College
Karen Dolce  2002-2005  ACVP Diplomate  Independent lab

Select Refereed Publications:


Grants Funded (last 3 years):