AUGUST 12 – 14, 2011
HYATT REGENCY AT THE ARCH
ST. LOUIS, MISSOURI

Conference Program

Friday, August 12th

1:30 PM — Conference Welcome
Lee Arnold; CHF Chairman of the Board
Steve Remspecher; Nestlé Purina PetCare

1:40 PM — History of Veterinary Medicine and Canine Research
Donald F. Smith, DVM; Cornell University

2:30 PM — Dog Genetics and the Mammalian Mind
Mark Neff, PhD; Van Andel Research Institute

3:15 PM — *Afternoon Break*

3:30 PM — Coping with Stress: Nutritional Approaches to Enhanced Immune Function
Arleigh Reynolds, DVM, PhD; Nestlé Purina PetCare

4:15 PM — Advances in Diagnosis and Treatment of Canine IBD
Albert Jergens, DVM, PhD; Iowa State University

5:00 PM — Canine Degenerative Myelopathy: A Translational Medicine Approach to Amyotrophic Lateral Sclerosis – Lou Gehrig's Disease
Joan Coates, DVM; University of Missouri, Columbia

7:30 PM — *Coffee and Dessert*
Please join us for coffee, dessert and a cash bar. Mingle with fellow participants and meet the conference speakers.

Saturday, August 13th

7:00 AM — *Breakfast*

8:00 AM — Morning Announcements
Lee Arnold; CHF Chairman of the Board

8:05 AM — Recent Progress in Molecular Genetics of Cancer and Challenges Ahead
Jaime Modiano, VMD, PhD; University of Minnesota

9:00 AM — Comparative Cytogenetics of Cancer. Just how Human are our Dogs?
Matthew Breen, PhD; North Carolina State University

9:40 AM — Canine-derived Antibody Fragments for Targeted Therapy of Cancer
Nicola Mason, BVetmed, PhD; University of Pennsylvania

10:20 AM — *Morning Break*

10:40 AM — Canine Oncology Clinical Trials
Douglas Thamm, VMD; Colorado State University

11:20 AM — Vitamin D and Cancer
Rondo Middleton, PhD; Nestlé Purina PetCare

12:00 PM — *Lunch*

1:00 PM — Factors Influencing Development of New Veterinary Medicine Oncology Products
Karen Greenwood; Pfizer Animal Health

1:40 PM — Panel Discussion: Future of Canine Cancer Research and Treatment
Moderator: Duane Butherus, PhD

2:10 PM — *Afternoon Break*

2:25 PM Parent Club Health Information and the AKC Breeder of Merit Program
Susan LaCroix Hamil; CHF Director & AKC Delegates Health Committee

2:40 PM What We Know about the Inheritance of Dilated Cardiomyopathy, Arrhythmogenic Right Ventricular Cardiomyopathy and Subaortic Stenosis in the Dog
Kathryn Meurs, DVM, PhD; North Carolina State University

3:20 PM September 11 - Tens Years Later for the Search and Rescue Dogs
Cynthia Otto, DVM, PhD; University of Pennsylvania

4:30 PM Conference Dinner at Purina Event Center

Sunday, August 14th

7:00 AM *Breakfast*

7:30 AM Veterinary Student Session
Eddie Dzuik, MBA; OFA COO
7:30 - 8:15 Presentations
American Kennel Club - Debra Bonnefond
AKC Canine Health Foundation - Erica Kitchen
8:15 - 9:00 Round Table Discussions

8:00 AM CHF & Grant Sponsors – Working Together for a Healthier World for Dogs
Christine Haakenson, PhD; CHF CSO

9:00 AM Introductions to BreakOut Discussions
Three rounds of breakout sessions allow attendees to learn about the topics that interest them the most and provide discussion opportunities.

9:15 AM BreakOut 1
1. Search and Rescue Working Dogs
2. CHF Grant Process
3. Canine Nutrition
4. Fundraising Techniques & Ideas
5. Development and Use of Canine Health Surveys
6. Purina Parent Club Partnership Program
7. Research Advantages of the Purebred Dog
8. Comparative Medicine: Man's Best Friend

10:00 AM *Morning Break*

10:30 AM BreakOut 2
1. Financing Canine Health Research
2. Canine Health Information Center (CHIC) and DNA Repository
3. Orthopedic Foundation for Animals
4. Nutritional Needs of Sporting Dogs
5. The Research Cycle: Bench to Clinic
6. Challenges of Veterinary Pharmaceuticals
7. Genetic Tests: How to Interpret Results and Incorporate Them Into Your Breeding Program
8. Research Advantages of the Purebred Dog
9. Comparative Medicine: Man's Best Friend

11:15 AM Switch Breakouts

11:30 AM BreakOut 3
1. Fundraising Techniques & Ideas
2. Canine Health Information Center (CHIC) and DNA Repository
3. Development and Use of Canine Health Surveys
4. The Research Cycle: Bench to Clinic
5. Challenges of Veterinary Pharmaceuticals
6. Genetic Tests: How to Interpret Results and Incorporate Them Into Your Breeding Program
7. Research Advantages of the Purebred Dog
8. Comparative Medicine: Man's Best Friend

12:15 PM *Lunch*

The History of Veterinary Medicine and Canine Research

Donald F. Smith, DVM
Cornell University
Professor of Surgery

Biographical Sketch for Dr. Smith

Donald F. Smith, DVM, Dipl. ACVS, is Austin O. Hooey Dean Emeritus and Professor of Surgery, College of Veterinary medicine, Cornell University. Before becoming Dean in 1997, Smith was chair of the Department of Clinical Sciences and Associate Dean for Academic Programs at Cornell. A graduate of the Ontario Veterinary College, Dr. Smith is an elected member of the National Academy of Practices. Because of his interest in history and public policy, he has conducted interviews with veterinarians of all ages, but with a special affinity for those who graduated prior to 1940. His unique and inspiring collection of "lost voices of the profession" is available at www.vet.cornell.edu/legacy. Smith maintains a robust DVM student teaching schedule and serves as chair of Cornell's veterinary admission committee. Smith writes a blog on veterinary medicine and public policy that reaches readers in over 80 countries at www.veterinarylegacy.blogspot.com. He lectures nationally on topics ranging from "One Medicine, One Health" to the History of Veterinary Medicine.

Presentation Abstract

Modern veterinary medicine in the U.S. had its roots during the Civil War when over one million horses and mules were lost due to starvation, disease and trauma. During the ensuing half century, over 30 veterinary colleges sprang up in major cities, including five in New York and two in Chicago. Though most were proprietary, non university-based institutions, Harvard and New York University also developed veterinary colleges as outgrowths of their medical schools. The equine species was the prime interest in all of these schools.

With the precipitous decline of the horse in the early 1920s due to the advent of the internal combustion engine, all but one of the colleges in the major cities closed. The era of the land-grant, agricultural-oriented veterinary colleges that had also started during the last few years of the 19th century in places like Cornell, Ohio State and Washington State Universities prevailed, but veterinary medicine transitioned to a rural-based profession focusing primarily on farm animals, public health, and working horses.

Though dogs and cats became more important as pets in the early decades of the 20th century, their medical care remained a low priority for both academic and private practice veterinarians. Most of the veterinary research in the first half of the 20th century was related to diseases of livestock, poultry and horses, public health and zoonotic diseases.

Physicians, meanwhile, used dogs to study comparative physiology and pathology and to develop surgical techniques for use in people. Aseptic surgical techniques were used in medical schools on their dog colonies several decades before they became commonplace in veterinary colleges. Canine research derived from medical schools in the pre-WWII era crept into veterinary clinical medicine, complemented by new techniques developed by private practitioners and the occasional faculty clinician staffing companion animal hospitals.

Following WWII, a second wave of land-grant veterinary colleges was established and returning GI's and their families began to demand better veterinary services for the growing number of pets that began to populate cities and suburbia. Clinical specialties and board certification starting in the mid 1960s met a critical need in the provision of advanced individual pet care, as well as providing an academic infrastructure for companion animal research. Because very few veterinary colleges were located in major metropolitan areas, specialty hospitals like the Animal Medical Center (New York City) and Angell Memorial Hospital (Boston) became urban centers for advancing canine medicine and research.

Federal and state funding for veterinary research remained largely restricted to agriculture animals and diseases of public health importance, however, and companion animal-oriented veterinary scientists funded canine research on private donations or "piggy-backed" to NIH research protocols that used dogs and other animals to study human disease. It was not until the last two decades of the 20th century that major foundations, corporations and individual donors began to devote substantial resources for companion animal research.

Veterinary medicine today is still a small profession with insufficient political influence to manifest robust financial support for companion animal research. Ironically, despite the fact that over 80% of our veterinarians are involved in some aspect of companion animal medicine, over 80% of government-sponsored research goes to diseases of production and farm animals, and to public health.

Sadly, the increasingly acknowledged human health benefits that accrue to people who have pets as part of their family structure are not considered worthy of funding by the federal government. Ironically, this occurs at a time when the soaring cost of human health can be modulated by better understanding the role of pets play in reducing costly physical, mental and emotional conditions in people. The great tragedy of the 2010 federal health care bill is that animals and veterinary medicine remain estranged in the public policy arena from people and human medicine.

In addition to promoting the human-animal bond, contemporary canine research has taken an enormous step forward with the advent of molecular biology and genetics, and the elucidation of the canine and human genome. Not only does this allow researchers and breeders to focus on genetically-based diseases and conditions for both prevention and therapy, it also allows individual animal testing and broadly-based screening of breeding lines to improve phenotype in succeeding generations. The homology between an increasing number of recognized canine and human conditions is another important byproduct of the genome, allowing us to analyze and interpret DNA from individual animals with spontaneously-occurring diseases compared to normal dogs from breed-specific cohorts.

Infectious diseases that were so miraculously prevented with the development of a wide range of vaccines and anti-parasitic agents starting in the middle decades of the 20th century remain a serious problem in dogs as new diseases (or variants of former diseases) emerge. That some of these represent potent zoonotic threats to human health (especially for an increasing immune-compromised population) lends further credence to the need for advancing the theme of comparative medicine in public and private research funding.

Recommended References:

Smith, D.F. 150th Anniversary of Veterinary Education and the Veterinary Profession in North America. J Vet Med Educ 2010 37(4) 317-327.

Smith, D.F. 150th Anniversary of Veterinary Education and the Veterinary Profession in North America: Part 2: 1940-1970. J Vet Med Educ 2011 38(1) 84-99.

Smith, D.F. 150th Anniversary of Veterinary Education and the Veterinary Profession in North America: Part 3: 1970-2010. J Vet Med Educ 2011. In press.

www.veterinarylegacy.blogspot.com – a blog devoted to history, education and public policy issues in veterinary medicine.

Dog Genetics and the Mammalian Mind

Mark W. Neff, PhD
Van Andel Research Institute (VARI), Translational Genomics Research Institute (TGen)
Scientific Investigator
Director, Center for Canine Health and Performance

Biographical Sketch for Dr. Neff

Mark Neff is Associate Professor at two non-profit institutions, the Van Andel Research Institute (VARI) and the Translational Genomics Research Institute (TGen), and has served as Director of the Program in Canine Health and Performance aligned across both institutes since 2009. He received his Ph.D. in classical genetics in 1993 from the University of Virginia where he studied the cellular controls that guard against cancer. Upon completing his graduate work, he received a Human Genome Distinguished Postdoctoral Fellowship in 1993 to train in the laboratory of Dr. Jasper Rine at UC Berkeley where he participated in mapping the genome as part of the original Dog Genome Project. He moved to the School of Veterinary Medicine at UC Davis in 2003. As Associate Director of the Veterinary Genetics Laboratory at UC Davis, he and colleagues identified genes contributing to a variety of traits and diseases. At VARI and TGen, Dr. Neff's laboratory currently studies genes (1) contributing to breed predilections for cancer histology, (2) influencing canine anxiety and compulsivity disorders, and (3) establishing breed-specific instincts, such as pointing and sheepherding.

Presentation Abstract

The 1990s were declared "The Decade of the Brain" by an act of congress. Although we learned a great deal about the physical properties of neurons and how neuronal networks are formed, there remain profound gaps in our understanding of how variation in these networks actually shapes behavior. The lack of a basic understanding of brain function has meant that patients suffering from mental illness and psychiatric disease continue to have overwhelming unmet needs. Dogs also suffer from mental illness and psychiatric diseases, such as compulsive trancing, impulsive rage, and idiopathic noise phobias. Such disorders are difficult to diagnose and challenging to treat. Breakthroughs will come from elucidating the molecular etiologies of these disorders, but a full understanding of the 'breaks' in the central nervous system (CNS) will also require studies of so-called 'normal' behavior. The domestic dog is unique in that breeds demonstrate inherited predispositions for temperaments, tendencies, and action patterns. Revealing the genes responsible for these traits will also shed light on rules governing the mammalian mind, and thus are equally important for one treating both human and canine patients suffering from complex disorders of the CNS.

Coping with Stress: Nutritional Approaches to Enhanced Immune Function

Arleigh Reynolds, DVM, PhD, DACVN
Senior Research Scientist
Nestle Purina Research
Salcha, AK

Biographical Sketch for Dr. Reynolds

Dr. Arleigh Reynolds received a veterinary degree in 1986 and a doctoral degree in nutrition and biochemistry in 1992 from Cornell University in Ithaca, N.Y. Prior to becoming a Nestlé Purina Nutrition Scientist in 1998, Reynolds researched a variety of feline and canine nutrition and performance topics. Among the canine topics he studied were: the effect of training on thyroid function in sled dogs; hydration of racing sled dogs; the effect of fat intake on an animal's maximum capacity to metabolize oxygen; the effect of post-exercise carbohydrate supplementation on muscle glycogen repletion; and protein requirements in working dogs.

Presentation Abstract

It is well established that travel and competition result in an increased incidence of illness in professional human athletes. Changes in diet, environmental conditions, and exposure to novel pathogens may all play a role in this syndrome. The role of exercise varies with intensity and duration. Low to moderate exercise has been shown to enhance immune function, while extreme exercise compromises immune function. We have examined the effect of every day naturally occurring stressors such as exercise and travel on immune function in dogs. We have also measured the effects of nutrition on the immune system's response to these stressors. Studies examining the immune response to dietary supplementation with probiotics, egg and milk biologics, and antioxidants will be reviewed and discussed. Application of these principles to athletic, show and pet dogs will also be discussed.

Advances in Diagnosis and Treatment of Canine IBD

Albert Jergens, DVM, MS, PhD
Iowa State University
Professor, Veterinary Clinical Sciences

Biographical Sketch for Dr. Jergens

Dr. Jergens' clinical interests include: canine/feline gastroenterology, GI endoscopy, clinical trials, inflammatory bowel diseases, and host-microbiota interactions mediating intestinal diseases. Jergens' basic science research interests include: animal models of intestinal inflammation, mucosal immunology, and the intestinal microbiota in health and disease.

Presentation Abstract

Canine inflammatory bowel disease (IBD) denotes a heterogeneous group of idiopathic, chronic, relapsing inflammatory disorders of the gastrointestinal tract that are immunologically-mediated. While their exact etiologies remain unknown, research results suggest that interplay between genetic factors and enteric bacteria are crucial for disease development, owing to abnormal host responses directed against the commensal microbiota. Key clinical signs include vomiting, diarrhea and weight loss, and histopathologic lesions of inflammation may involve the stomach, small intestine, or colon. Recent advances in molecular tools, disease activity indices, and biomarker development now permit objective assessment of IBD severity at diagnosis and in response to various therapies. Treatment of IBD involves both dietary and pharamacologic interventions as well as therapeutic manipulation of the enteric microbiota through the use of antibiotics, prebiotics, and probiotics. This presentation will highlight recent research advances in canine IBD and offer insight into future research directions.

Relevant Publications:

Jergens AE, Crandell J, Morrison JA, et al. Comparison of oral prednisone and prednisone combined with metronidazole for induction therapy of canine inflammatory bowel disease. J Vet Intern Med 2010; 24:269-277. PMID: 20051005

Suchodolski JS, Xenoulis PG, Paddock CG, Steiner JM, Jergens AE. Molecular analysis of the bacterial microbiota in duodenal biopsies from dogs with idiopathic inflammatory bowel disease. Vet Microbiol; 2010 142:394-400. PMID: 19959301

Washabau RJ, Day MJ, Willard MD, Hall EJ, Jergens AE, Mansell J, Minami T, Bilzer TW; WSAVA International Gastrointestinal Standardization Group. Endoscopic, biopsy, and histopathologic guidelines for the evaluation of gastrointestinal inflammation in companion animals. J Vet Intern Med 2010; 24:10-26. PMID: 20391635

Simpson KW, Jergens AE. Pitfalls and progress in the diagnosis and management of canine inflammatory bowel disease. Vet Clin North Am 2011; 41:381-398.

Jergens AE, Simpson KW. Inflammatory bowel disease in veterinary medicine. Frontiers in Biosciences; in press.

Canine Degenerative Myelopathy: A Translational Medicine Approach to Amyotrophic Lateral Sclerosis – Lou Gehrig's Disease

Joan R. Coates, DVM, MS
University of Missouri
Professor, Veterinary Neurology & Neurosurgery

Biographical Sketch for Dr. Coates

Dr. Joan Coates is a Full Professor in the Department of Veterinary Medicine and Surgery at the College of Veterinary Medicine of the University of Missouri. She received her BS degree in General Agriculture in 1987 and DVM degree in 1990 from the University of Missouri. She then went on to a small animal rotating internship at Texas A&M University and then completed a residency in neurology and neurosurgery at Auburn University where she also acquired Master's of Science degree in neurosurgery in 1994. Since, she has served on the faculty at the University of Georgia from 1994 to 1997 and at Texas A&M University from 1997 to 2003 before returning as a faculty member to the University of Missouri. She is board-certified in neurology by the American College of Veterinary Internal Medicine. She has served on a number of committees within the ACVIM and is currently serving as Secretary for the Neurology Specialty. As a clinician, she is Service Leader for the Neurology and Neurosurgery Service and Co-Director for the Physical Rehabilitation Program at the University of Missouri Veterinary Medical Teaching Hospital. As a researcher, she is a member of the Comparative Neurology Program at the University of Missouri which explores the impact of genetics in developmental and degenerative diseases of the nervous system. Her main research focus involves the study of canine degenerative myelopathy. She most recently has co-authored with Drs. Mike Lorenz and Marc Kent the *Handbook of Veterinary Neurology 5th edition*.

Presentation Abstract

Canine degenerative myelopathy (DM) is an adult onset neurodegenerative disease that occurs in many breeds. First described by Averill in 1973, canine DM is a spontaneously occurring, adult-onset, progressive disease that leads to paralysis and death. Most dogs are at least 8 years old before they show clinical signs. The initial clinical signs are upper motor neuron asymmetric spastic weakness and general proprioceptive ataxia in the pelvic limbs, which subsequently progress to paraplegia. Dog owners often elect euthanasia within a year of diagnosis. When euthanasia is delayed, lower motor neuron signs emerge as ascending tetraparesis, flaccid paralysis, widespread muscle atrophy, difficulty swallowing and inability to bark. A definitive diagnosis of DM can only be accomplished postmortem. Histopathologically, DM is characterized by axonal and myelin degeneration and astroglial (sclerosis) proliferation that is consistently most severe in the dorsal portion of the lateral funiculus within the middle to lower thoracic region. We have additionally documented denervation atrophy in muscle (amyotrophy), nerve fiber loss with axonal degeneration and loss in myelinated fibers of peripheral nerves. DM has been histopathologically documented in over 18 different breeds of dogs.

Recently, we identified a *c.118G>A* transition in the superoxide dismutase 1 gene (*SOD1)* that predicts an E40K missense mutation in Pembroke Welsh Corgis with DM suggesting that DM is similar to some forms of human amyotrophic lateral sclerosis (ALS – Lou Gehrig's disease). We additionally showed a highly significant association between homozygosity for E40K and the DM phenotype in Pembroke Welsh Corgis and in four other dog breeds: Boxer, Chesapeake Bay Retriever, German Shepherd Dog,

and Rhodesian Ridgeback. In addition, we demonstrated that as in human ALS, cytoplasmic aggregates that bind anti-SOD1 antibodies were present in all spinal cords of *SOD1:c.118A* homozygotes with DM. We continue to assess the occurrence and clinical relevance of the E40K mutation in privately owned dogs. We have currently genotyped 18,564 dogs that represent over 200 different breeds at *SOD1:c.G118A* and found the *SOD1:c.118A* allele in 109 breeds or about half the breeds tested. Its frequency exceeds 70% in three breeds: the Wire Fox Terrier, Pembroke Welsh Corgi, and Boxer. Since not all *SOD1:c.118A* homozygotes develop clinical signs, DM appears to be an incompletely penetrant autosomal recessive disease; whereas most human *SOD1* mutations cause dominant forms of ALS. However, we have identified several dogs with signs of DM that are heterozygous for the E40K mutation, suggesting that this mutation may result in a disease phenotype that also shows dominant inheritance. Thus, although considered rare, it is possible that carrier dogs may develop DM.

Mutations in the superoxide dismutase *SOD1* gene, which encodes cytosolic Cu/Zn superoxide dismutase are most commonly associated with the inherited form of ALS (Familial ALS – fALS). The etiology of the more common non-familial ALS (sporadic ALS – sALS), which accounts for 90% of ALS is largely unknown. Since the initial discovery in 1993 that mutations in *SOD1* could cause ALS, more than 145 *SOD1* mutations have been identified in ALS patients (http://alsod.iop.kcl.ac.uk/) accounting for approximately 20% of fALS cases and about 2% of all cases. ALS in people is characterized by loss of motor neurons causing stiffness and slowing of muscle movements, difficulty speaking and swallowing, muscle atrophy and severe weakness. Patients typically die within 3-5 years secondary to failure of respiratory muscles. The distribution of lesions and clinical disease progression in DM is similar to that reported for the UMN-onset ALS.

Currently, there are no effective treatments for ALS or DM. The only currently FDA-approved drug for therapy in ALS, riluzole, has shown marginal effects at slowing disease progression. Therapeutic interventions that appeared promising when tested in rodent ALS models have failed to ameliorate signs of ALS in people. Spontaneous canine models of diseases have played a key role in developing therapies for a number of disorders in people and other animals. Moreover, canine disease models such as DM offer a ready clinical population on which therapies can be evaluated in an environment that closely mimics human clinical trials. Therefore, development of the canine model could prove to be a major breakthrough in the assessment of therapeutic interventions for ALS. When translating therapies from animals to people and vice-versa, sensitive and objective disease markers shared across species are necessary to diagnose and monitor disease progression so that therapeutic efficacy can be accurately predicted. Potential biomarkers for DM are being evaluated in neural tissue morphometry, cerebrospinal fluid, gait, imaging and electrophysiologic studies, and genome-wide association mapping.

Recent Progress in Molecular Genetics of Cancer and Challenges Ahead

Jaime F. Modiano, VMD, PhD
University of Minnesota
Director, Animal Cancer Care and Research Program, College of Veterinary Medicine
Perlman Professor of Oncology and Comparative Medicine, Veterinary Clinical Sciences

Biographical Sketch for Dr. Modiano

Jaime Modiano hails from Mexico City, where he graduated from the baccalaureate program at Colegio Columbia. He did undergraduate work in Biomedical Sciences at Texas A&M University in College Station, TX before moving on to veterinary school at the University of Pennsylvania in Philadelphia. He completed his veterinary training and PhD in Immunology through the Veterinary Medical Scientist Training Program at Penn, followed by a residency in Veterinary Clinical Pathology at Colorado State University in Fort Collins, CO and a post-doctoral fellowship at the National Jewish Center for Immunology and Respiratory Medicine in Denver, CO. His first faculty appointment as Assistant Professor was in the Department of Veterinary Pathobiology at Texas A&M University. Dr. Modiano then returned to Denver as Associate Professor of Immunology at the School of Medicine and Full Member of the Cancer of the University of Colorado, Denver (Health Sciences Center) and also held Scientist and Senior Scientist appointments at the AMC Cancer Research Center and Foundation. In July of 2007, Dr. Modiano joined the College of Veterinary Medicine, School of Medicine, and Masonic Cancer Center at the University of Minnesota, where he continues his research program as Alvin and June Perlman Endowed Professor of Oncology and Comparative Medicine.

Dr. Modiano also served as Director of Cancer Immunology and Immunotherapy for the Donald Monk Cancer Research Foundation; he is a partner at Veterinary Research Associates, LLC, a company focused on development and implementation of diagnostics for veterinary medicine and a founder/scientist at ApopLogic Pharmaceuticals, Inc, a biotechnology company focused on development of cancer therapeutics. His research program has had uninterrupted support from federal and private sources for 15 years, leading to co-authorship of more than 70 peer-reviewed scientific manuscripts, and more than 200 abstracts, presentations, and book chapters focused on various aspects of immunology, cancer cell biology, the genetic basis of cancer and applications of gene therapy.

Dr. Modiano is married to Dr. Michelle Ritt, a diplomate of the American College of Veterinary Internal Medicine who is a Clinical Associate Professor of Medicine at the University Of Minnesota College Of Veterinary Medicine. They share their home with two outstanding agility dogs - Logan, a Gordon setter and Quetzal, a German Shepherd Dog.

Presentation Abstract

The last decade has seen dramatic improvements in molecular genetic research of canine cancer. This includes new and improved diagnostic tests and approval of the first immune-based cancer therapy (ONCEPT, Merial canine melanoma vaccine) and the first targeted small molecule inhibitor (Palladia, Pfizer c-Kit inhibitor for treatment of mast cell tumors). There also has been significant progress defining breed-associated cancer susceptibility. Our efforts have focused on defining the role that “breed” plays not only on the frequency of tumor occurrence, but also on tumor behavior. At the same time, we have dedicated considerable effort to understand the mechanisms that drive tumor behavior as a means to improve our diagnostic precision, our prognostic capacity, and the development of new therapies. To help achieve these goals, AKC CHF has supported eight research projects in our laboratory since 1998. These projects have allowed us to build a sample bank that has been used extensively by the research community worldwide (more than 40 investigators at more than 30 companies, universities, and research institutes in seven countries and four continents). The projects also have formed a robust foundation for clinical translation, verifying the notion that oftentimes tumors are as unique as patients and we must beware of generalizations and oversimplification. This presentation will underscore the differences that exist within tumors and among dog breeds, and illustrate how we have started to overcome these challenges to achieve our clinical goals.

Comparative Cytogenetics of Cancer. Just How Human Are Our Dogs?

Matthew Breen, PhD, CBiol, FSB
North Carolina State University, College of Veterinary Medicine
Professor of Genomics

Biographical Sketch for Dr. Breen

Dr. Matthew Breen completed his PhD in cytogenetics in 1990 and then spent two years as a Post Doc in Molecular Genetics at the UK Medical Research Council's Human Genetics Unit in Edinburgh, where he developed new techniques as part of the human genome project. Dr. Breen then spent four years working for the research arm of the Australian Thoroughbred industry, returning to the UK in 1996 where his laboratory developed molecular cytogenetics reagents, resources and techniques for application to canine genome mapping, comparative cytogenetics and cancer studies. In 2002 Dr. Breen relocated his laboratory to NCSU's College of Veterinary Medicine as part of its Genomics initiative. Since then his research interests have continued to focus on the genomics, genome mapping and the comparative aspects of canine cancer. He is leader of the Clinical Genomics Core of the Center for Comparative Medicine and Translational Research and co-Director of the Clinical Studies Core. Dr. Breen currently has a number of active grants from the AKC-CHF that are focused on the molecular cytogenetic evaluation of canine tumors.

Presentation Abstract

The application of genomics to canine biomedical research has resulted in significant advances as we strive to enhance the health and welfare of our companions. Over the past several years we have recruited tumor tissues and blood samples from hundreds of dogs presenting with a variety of cancers, as well as their family members. During the same period we generated a series of sophisticated molecular cytogenetic reagents and resources that complete the genomics 'toolbox'. Collectively these tools provide a robust means to interrogate tumor specimens for organizational changes to the genome, which lead to identification of genome regions and gene associated with cancer.

We have demonstrated the presence of numerous cytogenetic signatures associated with canine cancer subtypes and are using these to offer a more sophisticated means of tumor diagnosis. In addition we have begun to define genomic lesions that correlate with prognosis. For example, in our CHF-funded work with canine lymphoma we have developed a cytogenetic test that allows us to predict how long dogs diagnosed with lymphoma will respond to doxorubicin based chemotherapy. We have demonstrated previously that the chromosome changes we continue to observe in several canine cancers are shared with the corresponding cancers in humans. These data provide strong evidence for a shared pathogenetic origin of several cancers affecting both human and dog. Analysis of our data has revealed we are well on the way towards development of more sophisticated molecular sub-classification of canine (and maybe even human) cancers, a process that should facilitate the emergence of improved and tailored therapies. Comparing the molecular cytogenetics of recurrent changes in human and canine cancers is allowing us to refine key signatures to a subset that are shared, thus reducing the size of regions of interest. By considering the canine and human genomes in such a comparative context, we have identified that the genomic complexity of cancers may be less than human studies alone have suggested. An overview of these studies will be presented.

Overall these studies are advancing rapidly and indicate that the keys to unlocking some of nature's most intriguing puzzles about cancers may be found in the genome of the dog. Finding such keys in the dog will also lead to improved understanding of human cancers. For 15,000 years the dog has been man's best friend, in the 21st Century it is becoming increasingly evident that the dog is also man's best biomedical friend.

Canine-derived Antibody Fragments for Targeted Therapy of Cancer

Nicola Mason, BVetMed, PhD
University of Pennsylvania
Assistant Professor of Medicine & Pathobiology

Biographical Sketch for Dr. Mason

Dr. Mason graduated from the Royal Veterinary College, University of London and spent a year in private practice in Peterborough. She then performed a small animal medicine internship at the University of Bristol and a small animal internal medicine residency at the University of Pennsylvania's School of Veterinary Medicine. She went on to complete her PhD in Immunology at the University of Pennsylvania and performed a post-doctoral fellowship in the laboratory of Dr. Carl June at the Abramson Cancer Center, within the University of Pennsylvania.

Dr. Mason is currently an assistant professor of medicine at the University of Pennsylvania's School of Veterinary Medicine. Dr. Mason is a Diplomate of the American College of Veterinary Internal Medicine, she is the associate director of translational research at the school's Mari Lowe Comparative Oncology Center and the director of the PennVet Tumor Tissue Bank. Her research focuses on targeted therapies for the treatment of canine cancers with particular emphasis on lymphoma, osteosarcoma and hemangiosarcoma.

Presentation Abstract

Antibodies that target tumor cells or neutralize their growth factors have revolutionized the treatment of many different human cancers. However such targeted antibody therapy is not currently available in veterinary medicine. Humanized antibodies used to target human cancers cannot be used in dogs because they don't cross-react with dog tumor cells and they are rapidly destroyed by the dog. In order to develop targeted antibody therapies for use in our canine patients, we have developed a platform technology to generate libraries of canine-derived antibody fragments from the white blood cells or lymphocytes of dogs with cancer. We have previously shown that libraries of antibody fragments generated from dogs vaccinated against canine parvovirus contain antibody fragments that bind to canine parvovirus. These findings provide proof-of-principle that libraries of canine-derived antibody fragments re-capitulate the antibody repertoire of the dog. We have now utilized libraries, generated from the spleens of dogs with hemangiosarcoma, to isolate antibody fragments that bind and neutralize the biological activity of Vascular Endothelial Growth Factor (VEGF). VEGF is considered to be one of the major factors that stimulates the growth of blood vessels in and around tumors, which is necessary for the survival of the tumor. Neutralization of VEGF has led to prolonged overall survival times in human patients with several different types of cancers. These canine-derived VEGF-specific antibody fragments represent the first targeted biological antibody therapy that may be employed in dogs to retard the growth of a number of different tumor types including canine hemangiosarcoma. Furthermore, libraries generated from dogs with different tumor types such as osteosarcoma and lymphoma may contain novel antibody fragments that could be used to target these cancer types in the future.

Canine Oncology Trials

Douglas H. Thamm, VMD DACVIM
Colorado State University Animal Cancer Center
Associate Professor and Barbara Cox Anthony Chair in Oncology
Director of Clinical Research

Biographical Sketch for Dr. Thamm

Dr. Thamm is an Associate Professor and Barbara Cox Anthony Chair in Oncology at the Colorado State University Animal Cancer Center, within the College of Veterinary Medicine and Biomedical Sciences. He is also a member of the Developmental Therapeutics Section of the University of Colorado Comprehensive Cancer Center and the Cell and Molecular Biology Graduate Program at Colorado State University. Dr. Thamm received his Bachelor's and V.M.D. degrees from the University of Pennsylvania. He completed a Residency in Medical Oncology at the University of Wisconsin, and was a researcher there for 5 additional years before joining the faculty at CSU in 2004. He is the author of over 65 peer-reviewed publications and was Oncology Section Editor for the most recent edition of Kirk's Veterinary Therapy. His clinical and research interests include novel targeted therapies for animal and human cancer and ways to integrate these therapies with existing treatment.

Presentation Abstract

What is a clinical trial?

Although there are many definitions of clinical trials, they are generally considered to be health-related research studies that follow a pre-defined protocol. These can include both interventional and observational types of studies. Interventional studies are those in which the research subjects are assigned by the investigator to a treatment or other intervention, and their outcomes are measured. Observational studies are those in which subjects are observed and their outcomes are measured by the investigators.

Why are clinical trials conducted in dogs with cancer?

One obvious reason is to investigate new and hopefully better ways to diagnose, treat and monitor cancer in dogs. However, many naturally occurring cancers in pet animals closely resemble human cancer and provide meaningful systems for cancer research to benefit both species. Thus, sometimes therapies are studied in dogs to provide important information about whether this form of treatment might be appropriate for testing in humans.

What are the different types of clinical trials?

- Treatment trials test new treatments, new combinations of drugs, or new approaches to surgery or radiation therapy.
- Prevention trials look for better ways to prevent disease in patients that have never had the disease or to prevent a disease from returning. These approaches may include medicines, vaccines, vitamins, minerals, or lifestyle changes.
- Diagnostic trials are conducted to find better tests or procedures for diagnosing a particular disease or condition.
- Screening trials test the best way to detect certain diseases or health conditions.
- Quality of Life trials (or Supportive Care trials) explore ways to improve comfort and the quality of life for individuals with illness.

What are the phases of clinical trials?

Clinical trials are conducted in phases. The trials at each phase have a different purpose and help scientists answer different questions:

In Phase I trials, researchers test a new drug, drug combination or treatment in a small group of patients, often with different types of cancer for the first time to evaluate its safety, determine a safe dosage range, and identify side effects. Typically, a very low dose of a new drug is tested initially, and if there are no side effects, that dose is gradually increased in additional patients.

In Phase II trials, the new study drug or treatment is given to a larger group of patients, usually with the same type of disease, to see if it is effective and to further evaluate its safety.

In Phase III trials, the new study drug or treatment is given to still larger groups of patients to confirm its effectiveness, monitor side effects, compare it to commonly used treatments, and collect information that will allow the treatment to be used safely. These trials are usually the types of trials necessary for the regulatory approval of a new drug or treatment by agencies such as the FDA or USDA.

In Phase IV trials, post marketing studies delineate additional information including the drug's risks, benefits, and optimal use.

When are clinical trials appropriate for a pet?

Some clinical trials, for example, studies that involve simple procedures such as blood collection or special handling of tissues to be removed at surgery, may be appropriate for any dog with cancer. Participation in therapeutic trials with new drugs or treatments, especially early-phase trials, needs to be considered more carefully, as the effectiveness of these treatments is generally not as well known, and potential toxicity may not be as well established. Questions to ask when weighing the decision about whether to participate in a clinical trial include:

- What is the "standard of care" for treatment of my dog's disease, and how well does it work?
- Is this a disease that could be effectively treated with a simple surgery? If so, is it appropriate to do something else?
- How expensive is the "standard of care"?
- Unfortunately, sometimes the standard of care therapy may be unaffordable for a given owner. In these cases, considering an alternative such as participation in a clinical trial may mean the difference between a pet receiving an investigational therapy or no therapy.
- Are there other treatment alternatives besides the "standard of care", and how well do they work? How expensive are they?
- How much is known about the safety and effectiveness of the treatment being studied for my dog's cancer?

How can I find information about available clinical trials for dogs with cancer?

There are multiple websites that list ongoing clinical trials for pets with cancer. The most frequently updated and comprehensive is the site managed by the Veterinary Cancer Society.

http://www.vetcancersociety.org/clinical-research.html

Since the majority of (but not all) clinical trials are conducted at Veterinary Teaching Hospitals associated with Universities, making contact with the nearest University Veterinary Teaching Hospital may provide a simple way to find out about trials available in your area.

Vitamin D and Cancer

Rondo P. Middleton, PhD
Nestlé Purina Research
Senior Research Scientist, Pet Care Basic Research

Biographical Sketch for Dr. Middleton

Dr. Rondo P. Middleton completed his PhD in biochemistry in 1999 at the University of California, Riverside where he studied 1,25(OH)2 vitamin D3's ability to regulate gene expression at the transcriptional, translational and post-translational levels. He was hired by Ralston Purina as a research scientist in 1998. Dr. Middleton is currently a senior research scientist at Nestlé Purina Research Center where his research interests include osteoarthritis, cancer, aging, weight management, among others. Most of his work focuses on the molecular aspects (gene expression, metabonomics, systems biology) supporting these health and disease areas.

Presentation Abstract

Since its discovery as a molecule necessary for proper calcium and phosphate metabolism, vitamin D, and more specifically the hormonally active form, 1,25-dihydroxyvitamin D_3 (calcitriol), has become known as an important player in many other biological systems. These areas include cancer, heart disease, autoimmune diseases, and skin disorders, among others[1]. Physiological responses to calcitriol are mediated through the vitamin D receptor (VDR). The VDR elicits its actions by binding to specific regions on DNA and promotes or inhibits the expression of vitamin D-responsive genes. It was the discovery that the VDR was present in many tissues not involved in calcium homeostasis that led to the finding of the pleotropic actions of calcitriol.

Research has shown that higher levels of vitamin D in the blood are associated with reduced incidence and recurrence, and greater survival in various types of cancers. The expression of many genes encoding for enzymes involved in the metabolism of vitamin D, as well as other proteins regulated by vitamin D, are altered in cancer. These vitamin D-responsive genes and their respective proteins affect many processes involved in cancer. These include antiproliferation, pro-differentiation and pro-apoptotic effects[2]. We have previously shown that calcitriol and some associated vitamin D analogs can decrease the proliferation and induce differentiation in canine cancer SCC 2/88 cells *in vitro*[3].

In order to further understand the molecular mechanisms associated with calcitriol's antiproliferation and pro-differentiation effects, we recently investigated the gene expression changes involved in canine transitional cell carcinoma cells in response to calcitriol and some of its associated analogs. Additionally, due to the involvement of reactive oxygen species (oxidative stress) in cancer, we have investigated the role of antioxidant enzymes in these canine cancer cells. Antioxidant enzymes appear to interact with the identified differentially expressed genes and show beneficial modulation of key cancer processes in our study.

[1]DeLuca, H.F and Plum, L.A. Vitamin D, disease and therapeutic opportunities. *Nature Reviews Drug Discovery*, 9:941-955, 2010.

[2]Deeb, K.K., Trump, D.L. and Johnson, C.S. Vitamin D signaling pathways in cancer: potential for anticancer therapeutics. *Nature Reviews Cancer*, 7:684-700, 2007.

[3]Kunakornsawat, S., Rosol, T.J., Capen, C.C., Middleton, R.P., Hannah, S.S. and Inpanbutr, N. Effects of $1,25(OH)_2D_3$, EB1089, and Analog V on PTHrP production, PTHrP mRNA expression and cell growth in SCC 2/88. *Anticancer Research*, 21:3355-3364, 2001.

Factors Influencing Development of New Veterinary Medicine Oncology Products

Karen Greenwood, BSc
Pfizer Animal Health
Director, Companion Animal Internal Medicine Unit

Biographical Sketch for Karen Greenwood

Karen Greenwood is currently employed at Pfizer Animal Health in Kalamazoo, Michigan having moved to the United States from England in November 2006. She received a BSc (Hons) in Biochemistry at Southampton University, England, in 1988, and then took a role with Pfizer Animal Health Veterinary Medicine Research and Development (VMRD) in the Fermentation Titer Improvement group. After two years she transferred to the VMRD Biology group to work on in vitro screen design and development, which was a major focus for the next 16 years along with leadership of teams seeking new treatments for various diseases states, in particular antiparasitics. She moved to Kalamazoo to lead the Companion Animal Discovery Unit, with responsibility for finding new treatments for diseases in dogs, cats and horses, from identification of potential targets through proof of concept. Karen enjoys the intricacies of drug discovery and development, and the opportunity to be able to turn cutting edge science into products that benefit pets and owners. She serves on the Grants Committee of the AKC Canine Health Foundation. Karen lives just outside Kalamazoo with her husband Sean, a synthetic chemist with Pfizer, and her two small children, Zoe and Hayley.

Presentation Abstract

The presentation will cover the process involved in identifying and registering new oncology treatments for canines and the factors that influence successful registration and launch. This will include observations on the challenge and complexity of development for agents specifically developed for pets versus off-label use of human health products, the MUMS (minor use, minor species) process as it relates to oncology, and the benefits of translational oncology for canine cancer patients.

What We Know About the Inheritance of Dilated Cardiomyopathy, Arrhythmogenic Right Ventricular Cardiomyopathy and Subaortic Stenosis in the Dog

Kathryn M. Meurs, DVM, PhD
North Carolina State University College of Veterinary Medicine
Professor and the Associate Dean of Research and Graduate Studies

Biographical Sketch for Dr. Meurs

Dr. Meurs is a Professor and the Associate Dean of Research and Graduate Studies at North Carolina State University College of Veterinary. She completed her DVM in 1990 at the University of Wisconsin – Madison and completed a small animal internship at North Carolina State University in 1991. She completed a Cardiology residency at Texas A&M University and is board certified from the American College of Veterinary Internal Medicine (Cardiology).

Dr. Meurs has a Ph.D. in Genetics from Texas A&M University and her areas of interest include familial aspects of cardiovascular disease, especially cardiomyopathy.

Presentation Abstract

Cardiomyopathy is a primary muscle disease that has been shown to be inherited in the dog as well as several other species. There are two common forms, dilated and arrhythmogenic.

Dilated cardiomyopathy is characterized by heart muscle dysfunction and enlargement of the heart chamber, particularly the left. Affected dogs may die suddenly or develop congestive heart failure as characterized by coughing and shortness of breath. There is no cure. It has been known to be inherited in the Newfoundland, Irish Wolfhound, Scottish Deerhound, Great Dane and Doberman pinscher, among other breeds. In North America, the most commonly reported breed is the Doberman pinscher and the largest number of studies have been done on this breed. In the Doberman pinscher the disease is inherited in an autosomal dominant mode and at least in some families is associated with a mutation in a gene involved in the energy metabolism of the heart. The cause of the disease in the other breeds is not known. In human beings there are now 20 different genes that cause the development of this disease. It is likely that there is more than 1 cause in the dog, and even in the Doberman pinscher as well. An important aspect of all cardiomyopathies is that they are impacted by variable penetrance, meaning that not all dogs that have the genetic cause will show the same severity of disease, some will show severe clinical signs, while others will remain free of symptoms there whole life.

Arrhythmogenic Right Ventricular Cardiomyopathy (ARVC) is a heart muscle disease characterized by cardiac arrhythmias that result in fainting or sudden death. It is most commonly observed in the Boxer dog, but has been observed in English Bulldogs as well. A deletion mutation has been identified in many affected Boxers. The mutation prevents the cells from holding together properly and this leads to cardiac arrhythmias and sudden death. As in other cardiomyopathies, variable penetrance exists meaning that not all dogs that have the mutation will have the same severity of disease. Although English Bulldogs also suffer from this disease they do not have this mutation.

Subvalvular Aortic Stenosis is one of the most common heart birth defects in the dog. It is known to be inherited in the Newfoundland, Rottweiler and Golden Retriever. Affected dogs can live comfortably with the mild form of the disease, but the severely affected dogs have an average life span of 2 years. We have some preliminary data that suggests that the disease may have a similar genetic cause in the Rottweiler and Golden Retriever but each breed may have additional genetic modifiers as well.

September 11 - Tens Years Later for the Search and Rescue Dogs

Cynthia M. Otto, DVM, PhD
University of Pennsylvania
Associate Professor, Critical Care
Director, Penn Vet Working Dog Center

Biographical Sketch for Dr. Otto

Dr. Otto, a board-certified emergency and critical care veterinarian, is currently a tenured associate professor of Critical Care at the University of Pennsylvania, School of Veterinary Medicine. She graduated from the Ohio State University, completed a rotating internship at the University of Pennsylvania and a residency in internal medicine and PhD in veterinary physiology at the University of Georgia. She obtained board certification in the American College of Veterinary Emergency and Critical Care (ACVECC). She served as Vice President of ACVECC for 2 terms and editor of the Journal of Veterinary Emergency and Critical Care for 5 years. She has been an attending veterinarian in the Ryan Veterinary Hospital Emergency Service since 1991. Dr. Otto has also been involved in disaster medicine as a member of the Pennsylvania Urban Search and Rescue Task Force 1 between 1994 and 2010 (including deployments to Hurricane Floyd and 9/11), and the Veterinary Medical Assistance Team-2 since 1999 (deploying to Hurricane Katrina). She has been monitoring the health and behavior of Urban Search and Rescue canines since October of 2001, through an AKC-CHF funded grant (now in its third renewal). She is the founder of the Penn Vet Working Dog Center and has started the AKC-CAR Detection Dog DNA bank. She has organized the PennVet Working Dog Conference in 2010, and the upcoming conference in Sept of 2011 and serves as the co-chair of the 10 year anniversary 9/11 Tribute to the Search Dogs and Veterinarians (www.findingoneanother.org). She is active in educating search dog handlers and members of the working dog community in canine care. She was named Pennsylvania's 2002 "Veterinarian of the Year" and received an Alumni Recognition Award in 2006 and the OSU Distinguished Alumnus Award in 2008 from the Ohio State University.

Presentation Abstract

September 11, 2001 was an unprecedented day in the history of the United States. In response to the terrorist attacks in New York, Washington DC and the downed plane in Pennsylvania, hundreds of search and rescue and other canine teams were deployed. During the deployments in New York, both at Ground Zero and at the Staten Island Landfill, and in Washington DC at the Pentagon, the health and well-being of the dogs was monitored. Remarkably, the dogs coped with the adverse conditions with minimal morbidity. The most commonly reported problems reported by handlers were cuts and scrapes, most of with were minor. Problems related to the intensive work included fatigue, weight loss and dehydration. Interestingly enough, respiratory problems were rare. The human responders have been plagued with chronic respiratory conditions, however based on the ongoing monitoring of the dogs, respiratory problems were minimal. In fact, there have been no systematic conditions that have been identified in deployed search and rescue dogs that did not also occur in control (non-deployed) search and rescue dogs. Although not associated with clinical disease, the review of the first 5 years of chest x-rays identified more heart abnormalities identified in the deployed dogs. Surprisingly, there was minimal lung pathology in both groups both on x-rays and at post mortem examination. Coming up on the 10 year anniversary 73% of the deployed dogs have died. Of the dogs that have died, the median age at death was 12.2 years in deployed dogs, whereas in the controls it was 11.7 years. In both groups cancer was common and confirmed in 40% of the deployed and 45% of the control dogs. We are following trends in types of cancer to determine if there are any differences between deployed and control; however the numbers are still low. The legacy of 9/11 has been an increased awareness of the important role that these dogs play and the need for continued research in behavior, genetics and sports medicine to enhance their capacity and safety. To that end, the Penn Vet Working Dog Center has been established at the University of Pennsylvania.

CHF & Grant Sponsors – Working Together for a Healthier World for Dogs

Christine Haakenson, PhD
AKC Canine Health Foundation
Chief Scientific Officer

Biographical Sketch for Dr. Haakenson

Dr. Christine Haakenson is the Chief Scientific Officer (CSO) for the AKC Canine Health Foundation and is responsible for the development and direction of the Foundation's research and education strategy to fulfill the mission of the Foundation; advancing the health of all dogs and their owners by funding sound scientific research and supporting the dissemination of health information to prevent, treat, and cure canine disease.

Dr. Haakenson graduated with a Bachelor of Science in Chemical Engineering from Cornell University and holds a Ph.D. in Biochemistry and Molecular Biology from Georgetown University. Her research experience includes investigations in the cellular mechanisms of polyphosphates by analyzing viability following DNA damage. The research focused on damage induced repair pathways and effects on human breast cancer.

Prior to returning to graduate school, Dr. Haakenson worked over five years at Accenture, a global management consulting company. Her experiences included business/technology consulting to optimize R&D business processes.

Dr. Haakenson works in collaboration with the Foundation's grants committee, external peer reviewers, principal investigators, board of directors and staff. The overall goal is to ensure that the Foundation funding is strategically and optimally applied.

Presentation Abstract

The relationship between the AKC Canine Health Foundation and Parent Clubs, breed health foundations, and other sponsor organizations is very important and is part of the Foundation's distinction. We all share the goal to improve the health and lives of dogs through funding scientific research. The focus of this presentation is on this relationship and providing information about available resources and tools for organizations and their designated CHF Health Liaison.

The CHF Health Liaison is a very important person in the Foundation's relationship with breed organizations and we want to ensure they have what they need to perform this role. This person is the breed organization member who functions as the main contact for communication with CHF regarding the health and wellbeing of their breed, research projects, and grant sponsorships. They are also critical in communicating back with their club about research discoveries, new programs, and canine health educational resources. Therefore, providing them with the information and tools they need is a major component to the relationship.

During this presentation, I will describe: 1) the grant sponsorship process; 2) what the Health Liaison should expect from the Foundation; 3) the tools and resources available including a soon to be released electronic newsletter just for Health Liaisons; and 4) where to find information on the CHF website. The CHF website offers a wealth of knowledge and is constantly being updated with the latest grants, research findings, and news and events, including 'Success Story' articles that highlight scientific discoveries and the latest podcasts to listen to your favorite researcher.

Along with the information that is always available on our website, the Liaison can always contact us at the Foundation for questions regarding administration of funds, identification of researchers, and sponsorship opportunities. We are also able to pull reports regarding DAF information, grant sponsorship history, grants seeking sponsorship, grants specific to a breed and/or disease, and more! We want to provide information and resources that you need to be successful in building your breed's health programs.

I also want to take this opportunity to introduce Samantha Wright who has joined the AKC Canine Health Foundation team as the new Program Manager. Ms. Wright comes to the Foundation with a life long love of dogs, especially for her family's breed, the Gordon Setter. Samantha graduated from University of New Hampshire with a degree in zoology which focused on animal physiology, genetics, and behavior as well as building interdisciplinary skills including communications and education. Samantha is excited about this new opportunity and is looking forward to increasing her interactions with the Fancy through dog club communications, health liaison relationships, presentations and dog events. Please email Samantha at sjw@akcchf.org or call her toll free at 888-682-9696 with any questions.

RESOURCE CONTENT

PRESENTATION SUPPORTING ARTICLES

GENETICS AND BREEDING

CANCER 101

USEFUL LINKS

Medical and behavioral surveillance of dogs deployed to the World Trade Center and the Pentagon from October 2001 to June 2002

Cynthia M. Otto, DVM, PhD, DACVECC; Amanda B. Downend, BA; James A. Serpell, PhD; Lisa S. Ziemer, VMD; H. Mark Saunders, VMD, MS, DACVR

Objective—To evaluate early medical and behavioral effects of deployment to the World Trade Center, Fresh Kills Landfill, or the Pentagon on responding search-and-rescue (SAR) dogs.

Design—Prospective double cohort study.

Animals—The first cohort included SAR dogs responding to the September 11, 2001, terrorist attacks (deployed), and the second cohort included SAR dogs trained in a similar manner but not deployed (controls). Enrollment occurred from October 2001 to June 2002.

Procedure—Dogs were examined by their local veterinarians; thoracic radiographs and blood samples were shipped to the University of Pennsylvania for analysis. Handlers completed medical and training histories and a canine behavioral survey.

Results—Deployed dogs were older and had more search experience than control dogs. Serum concentrations of globulin and bilirubin and activity of alkaline phosphatase were significantly higher in deployed dogs, independent of age and training. Despite significant differences in several blood parameters, values for both groups were within reference ranges. No pulmonary abnormalities were detected on radiographs, and no significant differences in behavior or medical history were detected between groups.

Conclusions and Clinical Relevance—Within the first year following the September 11 attacks, there was no evidence that responding dogs developed adverse effects related to their work. Mild but significantly higher serum concentrations of globulin and bilirubin and activity of alkaline phosphatase in deployed dogs suggested higher antigen or toxin exposure. These dogs will be monitored for delayed effects for at least 3 years. (*J Am Vet Med Assoc* 2004;225:861–867)

The attacks of September 11, 2001, in New York; Washington, DC; and Pennsylvania resulted in loss of life and destruction that, as an act of terrorism, had not been previously experienced on American soil. Part of the rescue and recovery response included an estimated 250 to 300 **search-and-rescue** (SAR) dogs that were used at 3 major sites.

The site with the greatest damage and requiring the most substantial response was termed ground zero in lower Manhattan, where the **World Trade Center** (WTC) towers and several buildings in the vicinity were destroyed. As a result, 2,829 people were killed,[1] including 343 rescue workers[2] and 1 working dog.[3] The draft report released from the National Center for Environmental Assessment (**Environmental Protection Agency's** [EPA's] national resource center for human health and ecological risk assessment) in October 2002[4] cited particulate matter, asbestos, metals, dioxin-like material, polychlorinated biphenyls, and volatile organic compounds as concerns for acute and potentially chronic complications secondary to deployment exposure. In addition, smoke from fires that burned until mid-December 2001 acted as an irritant and many potential toxins remain unidentified. Search dogs from around the country first arrived at this site on September 11, 2001, when these hazards were thought to be at their highest concentrations. Those dogs left by early October, whereas New York City Police dogs remained at the site well into 2002. The EPA draft report suggests that the contaminant concentrations at ground zero remained high into early 2002.

The second site, Fresh Kills Landfill, was closely linked to the WTC. Located on Staten Island, Fresh Kills was the largest active landfill in the world and had been accumulating refuse for 50 years until March 2001, when it officially closed.[5] The debris from the WTC was transported to Fresh Kills beginning on September 12, 2001, where it was sorted and searched for human remains. The human and canine responders were exposed to hazardous material from ground zero and the landfill itself. The constant sifting of the debris increased the potential for production of airborne particulate matter (mainly asbestos and aerosolized toxins). Monitoring for asbestos by the EPA, however, did not start until early October.[4] Human workers at this site were equipped with respirators and polyethylene suits; the dogs had no protection.

The third site was the Pentagon, where a Boeing 757 crashed into the side of the building, destroying a segment of the outer ring and killing 184 innocent people on the ground and in the airplane as well as 5 terrorists.[6] The public information regarding hazardous materials involved at this site is more limited; however, an EPA monitoring summary from October 9, 2001,

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reported only trace concentrations of asbestos, volatile organic compounds, and other chemicals in the air of the building work zone. In both the building work zone and debris sorting area, however, high concentrations of antimony and arsenic were found in ash and soot.[7] Human workers were required to wear respirators and protective clothing. Again, the dogs had no protection. The dogs were actively searching at the Pentagon from September 11 until September 29, 2001.

There are no prior studies to evaluate the potential long-term medical or behavioral effects of SAR activity in dogs after such large-scale urban disasters. The only study[8] of adverse effects of search activity in SAR dogs in the United States reported medical illness (eg, fatigue, change in appetite, vomiting, and diarrhea) and injuries (eg, foot or skin lacerations and ocular irritation) during and behavioral changes immediately after the 1995 search of the bombed Murrah Federal Building in Oklahoma City. Historically, the focus of long-term studies in humans has been on psychological rather than medical effects of disaster response. Following the September 11 attacks, human medical surveillance of the New York City rescue personnel was initiated. Availability of funding and presence of confounding factors limited these studies; however, the primary causes of morbidity to date have been pulmonary (increased airway reactivity)[9] and psychological sequelae.[a]

Monitoring the SAR dogs that responded to the September 11 attacks is important for 2 major reasons. First, if problems develop in these dogs, training techniques and preventive measures can be developed to safeguard them in the future. Second, as supported by investigations of military working dogs in Vietnam, working dogs may serve as sentinels for human health hazards.[10]

It was hypothesized that adverse medical consequences of searching at any of the 3 sites would result in abnormalities detected via hematologic analysis of blood, serum biochemical analyses, or thoracic radiographs, compared with findings in SAR dogs that were not deployed to these sites. Although many dog handler teams ceased SAR activities after the response to the Oklahoma City bombing and Duhaime et al[8] reported that 33% of the search dogs had subjective behavioral changes during the week after completion of the mission. In working dogs, there has never been an investigation of behavioral abnormalities consistent with **posttraumatic stress disorder (PTSD)** in humans. It was also hypothesized that deployed dogs inadequately prepared for this type and intensity of work would have increased prevalence of negative behavioral traits, compared with control dogs. Subsequent comparison of training history, work conditions, and other variables in the deployed dogs could then prove useful in defining risk factors for the development of adverse behavioral traits. Therefore, the purpose of the study reported here was to evaluate early medical and behavioral effects of deployment to the WTC, the landfill, or the Pentagon on responding SAR dogs.

Materials and Methods

Dogs and handlers—Handlers of SAR dogs that were deployed to the WTC, Fresh Kills Landfill, and Pentagon disaster sites were identified and contacted for enrollment in a health and behavioral study of their dogs that was funded for 3 years, with the anticipation of lifetime surveillance. Because there was no central registry or official record of responding search dog teams, handlers were identified in several ways. The **American Kennel Club (AKC)** shared the deployed handler list they generated, including those who deployed through the **Federal Emergency Management Agency (FEMA)** and several who self-deployed or deployed through a non-FEMA organization. Additionally, a national listing of FEMA-certified handlers was used to enroll nondeployed dogs as control dogs and identify additional deployed individuals who were not on the AKC list. Also, some handlers from the deployed and control groups contacted the research group to enroll after learning of the study through the media or other handlers.

The deployed cohort consisted of the dogs that worked at 1 of the 3 disaster sites. Dogs that did not deploy, but had similar background and training, constituted the control cohort.

Data collection—The deployed and control group handlers were contacted and enrolled through e-mail, telephone, or regular mail. The recruitment period began in October 2001 and ended in June 2002. A pet insurance company[b] initially agreed to donate a 1-year health insurance policy to handlers for dogs that were enrolled in the study, and this company subsequently extended these policies for the life of the deployed dog and the duration of the study for the control dogs. Consent forms were completed for participation in the study.

Samples—In addition to surveys, a subset of deployed and control handlers submitted blood samples and thoracic radiographs from dogs, which were obtained by their local veterinarians. Because of cost constraints, blood sampling and radiographs were only offered to FEMA-deployed dogs and those deployed to the landfill. The FEMA group was chosen because documentation of their training and work was readily available. The landfill group was chosen because of the small number of dogs deployed and the lack of FEMA team representation from this site. All costs associated with collection of these samples were covered by the study. Complete blood counts[c] and serum biochemical analyses[d] were performed by the Clinical Pathology Laboratory at the Matthew J. Ryan Veterinary Hospital at the University of Pennsylvania. Complete thoracic radiographs (right and left lateral and ventrodorsal or dorsoventral) were evaluated by 2 university radiologists, who were blinded to the study groups.

If any dog died during the study period, a full necropsy was requested. The necropsy protocol included gross examination performed by the attending veterinarian and specified samples shipped to the Michigan State University for histopathologic analysis.

Surveys—Information from handlers was collected via survey instruments. A predeployment and deployment health survey and behavior questionnaire were distributed beginning in October 2001.[e] The health survey requested the complete contact information for the handler and the veterinarian in charge of the SAR dog's care; the SAR dog's complete medical history, including any health problems that developed while deployed; information about the dog's activity, including date of arrival, specific site worked, and disposition during deployment; and information about the dog's SAR training, including length and frequency of such training.

The behavior questionnaire[f] was a recently developed instrument for measuring behavior and behavior problems in dogs. It was tested for reliability and validity on a sample of more than 2,000 companion dogs.[11] The questionnaire measured 11 behavior traits identified by factor analysis, including various forms of aggression and fear; separation-related behavior; excitability; trainability; predatory chasing; and attachment or

attention-seeking. In addition, the questionnaire included a list of 24 miscellaneous behaviors that have not yet been validated.

Statistical analyses—For continuous variables, the 2-sample *t* test (normally distributed) or Mann-Whitney test (nonparametric data) was used. For categoric variables, the χ^2 test was used. The relationship between multiple variables was evaluated by use of multivariable linear regression and data transformation as appropriate to achieve normality. To compare the effect of deployment site on hematologic and serum biochemical results, 1-way ANOVA was used. For data that could not be transformed to achieve normal distribution, Kruskal-Wallis ANOVA was used. Because none of these comparisons yielded significant ($P \leq 0.05$) differences, no post hoc testing was used. A power analysis was performed to determine the smallest detectable difference between deployed and control dogs for blood analysis results.[12,g,h] In a post hoc analysis of dogs that worked at the WTC, dogs were grouped according to arrival date for comparisons of blood values. Dogs that arrived on September 11 or 12 were compared with dogs that arrived on September 13 to 17, on September 18 to 24, or after September 24.

Results

Although there was no single central registry of all deployed dogs, 212 deployed handlers were identified and contacted; 101 (48%) handlers enrolled. However, when data collection was terminated for the first year of the study, only 97 health surveys and 95 behavioral surveys from 97 deployed handlers were collected and completed properly for inclusion in the data set. Of the 212 deployed handlers contacted, 111 declined to participate. Ten handlers provided explanations for nonparticipation, including lack of time to complete the study protocol (n = 3), disagreement with the nature of the study (2), dog was currently sick (3), dog died before handler could participate (1), and handler was uncomfortable with the postmortem requirement (1). Seven handlers declined participation without providing a reason, and 25 handlers failed to complete the protocol after agreeing to participate. The remaining handlers (n = 69) could not be reached via their contact information or did not respond to requests for participation. Of the 114 identified control teams, 59 (52%) handlers agreed to participate. At the close of the initial data collection period for this subset, health surveys and behavioral surveys had been collected from 55 controls and were completed properly for the data set.

Handlers were deployed with their dogs (n = 97) to the WTC (61), Pentagon (23), and Fresh Kills Landfill (13) for a median duration of 10, 12, and 7 days, respectively. The median age of deployed dogs was 5.0 years (interquartile range, 3.0 to 7.0 years); control dogs were a median age of 4.0 years (interquartile, range 2.0 to 6.0 years; $P = 0.02$). The deployed dog group consisted of 18 sexually intact males, 37 castrated males, 2 sexually intact females, and 39 spayed females. The control dog group included 8 sexually intact males, 23 castrated males, 4 sexually intact females, and 20 spayed females. The median weight for deployed dogs was 31.1 ± 7.1 kg (68.5 ± 15.6 lb), compared with 32.5 ± 7.7 kg (71.6 ± 17.0 lb) for control dogs ($P = 0.26$). The deployed dog group was composed mostly of German Shepherd Dogs (n = 32 [33%]) and Labrador Retrievers (28 [29%]). Other breeds represented in this group were Golden Retrievers (12 [12%]), mixed breeds (8 [8%]), Border Collies (7 [7%]), and Australian Shepherds (4 [4%]) and a Beauceron (1 [1%]), Belgian Tervuren (1 [1%]), Doberman Pinscher (1 [1%]), English Springer Spaniel (1 [1%]), Giant Schnauzer (1 [1%]), and Rottweiler (1 [1%]). The control dog group was predominantly composed of German Shepherd Dogs (n = 25 [45%]) and Labrador Retrievers (12 [22%]). Other control dog breeds included Airedale Terriers (2 [4%]), Australian Cattle Dogs (2 [4%]), Belgian Malinois (2 [4%]), Hovawarts (2 [4%]), and Golden Retrievers (2 [4%]) and a Belgian Tervuren (1 [2%]), Bloodhound (1 [2%]), Border Collie (1 [2%]), Louisiana Catahoula Leopard Hound (1 [2%]), mixed breed (1 [2%]), Newfoundland (1 [2%]), Rottweiler (1 [2%]), and German Shorthaired Pointer (1 [2%]). Neither the sex distribution nor the breed distribution was different between deployed dogs and control dogs ($P = 0.39$ and 0.30, respectively). Other characteristics of the dogs were tabulated (**Table 1**). The deployed dogs had more

Table 1—Variables (median [range]) associated with 97 search-and-rescue (SAR) dogs that were deployed to 3 sites after the September 11, 2001, terrorist attacks and 55 SAR dogs that were not deployed (control dogs).

Variable	Deployed dogs	Control dogs	*P* value
Years of training	5.0 (3.0–6.0; n = 96)	3.5 (1.5–6.0; 53)	0.01
Years active search experience	4.0 (2.0–6.0; 96)	2.5 (1.0–4.0; 51)	0.02
Formal training sessions per month	4.5 (3.1–8.0; 95)	8 (6–12; 51)	< 0.001
Informal training sessions per month	12.0 (8.9–22.1; 93)	14 (8–22; 51)	0.88
FEMA certified (No. reporting)	50 (96)	20 (54)	0.11
Type of search (No.)	Live only (38) Cadaver only (4) Dual (54)	Live only (27) Cadaver only (2) Dual (23)	0.38

FEMA = Federal Emergency Management Agency.

search experience than the control dogs. The deployed dogs enrolled in this study actively searched at the WTC between September 11 and October 6, 2001; at the Pentagon between September 11 and September 29, 2001; and at the Fresh Kills Landfill from September 17 until September 29, 2001. Most of the dogs arrived on site by September 12, 2001 (59% at the WTC and 43% at the Pentagon). During this initial period, airborne hazards were considered particularly high. For all sites, the median number of days spent searching was 10 (interquartile range, 8 to 12 days).

The majority (96%) of deployed dogs and control dogs were trained to find live victims; however, 56% of deployed dogs and 42% of control dogs were also trained to identify cadavers. Only 4 deployed and 2 control dogs were exclusively trained for cadaver search. There were no significant differences in the prevalence of previous medical ($P = 0.241$) or surgical ($P = 0.891$) problems between groups.

Blood samples for CBC and serum biochemical profiles were received from 72 deployed and 52 control dogs. Samples were shipped overnight. There was no difference in the incidence of hemolysis (22/72 vs 14/52) or lipemia (18/72 vs 13/52) in samples obtained from deployed versus control dogs, respectively. There were no significant differences in CBC results between groups except for lymphocyte and eosinophil concentrations, both of which were significantly lower in the deployed dogs ($P = 0.037$ and 0.026, respectively; **Table 2**). All mean or median values for both groups of dogs were within reference ranges. The power of the analysis was sufficient to identify any clinically important changes. To determine whether the work site influenced hematologic results, comparisons were made within the deployed group among the 3 sites; no significant differences were found.

Mean or median serum biochemical values were within reference ranges, although significantly higher serum concentrations of glucose, total protein, globulins, bilirubin, and cholesterol; higher serum activity of alkaline phosphatase; and lower concentrations of potassium, phosphorus, and albumin-to-globulin ratio were detected in the deployed dogs (**Table 3**).

To determine whether the higher globulin concentrations were related to the age or search history of the deployed dogs, a multivariable linear regression was performed. A log transformation of the globulin data was used to achieve normality. The primary predictor of increased globulins was deployment status. Age, years of training, or years of active search did not contribute significantly to the model. In addition, when 2 deployed dogs with clinical disease contributing to hyperglobulinemia (multiple myeloma and systemic aspergillosis) were removed from the analysis, the globulin concentration of the deployed dogs was still significantly ($P = 0.003$) higher than in the control dogs. No biochemical parameter was different among dogs deployed at the 3 sites.

In the post hoc analysis of dogs that worked at the WTC, blood values from dogs that arrived on September 11 or 12 (n = 26) were compared with values from dogs that arrived later (21; 4 dogs arrived from September 13 to 17, 14 dogs arrived from September 18 to 24, and 3 dogs arrived after September 24). Cholesterol concentration, lymphocyte concentration, and monocyte concentration were significantly higher in the early arrivals; however, as with any post hoc analysis, results should be interpreted with caution.

Although hemolysis was associated with significantly higher bilirubin concentration, there was no difference in degree of hemolysis between groups. Even when samples that were moderately or markedly hemolyzed were omitted, deployed dogs still had significantly higher serum bilirubin concentrations than control dogs. Furthermore, when a bilirubin value of 1.7 mg/dL was omitted for a dog with liver dysfunction (and an eventual diagnosis of systemic aspergillosis), deployed dogs still had significantly higher bilirubin concentrations than control dogs. Serum alkaline phosphatase activity was not related to bilirubin con-

Table 2—Results of CBCs in SAR dogs that were deployed to 3 sites after the September 11, 2001, terrorist attacks and SAR dogs that were not deployed (control dogs).

Variable	Deployed dogs (n = 72)	Control dogs (52)	Reference range
WBC ($\times 10^3/\mu L$)	6.53 (5.48–8.05)	6.86 (5.40–9.38)	5.30–19.8
RBC ($\times 10^6/\mu L$)	7.02 ± 0.721	7.03 ± 0.72	5.83–8.87
Hemoglobin (g/dL)	16.4 ± 1.6	16.5 ± 1.6	13.3–20.5
Hct (%)	49.0 ± 5.1	49.0 ± 5.3	40.3–60.3
MCV (fL)	70.0 ± 3.3	69.8 ± 3.4	62.7–75.5
MCH (pg)	23.5 ± 0.9	23.6 ± 0.7	22.5–26.9
MCHC (g/dL)	33.8 (33.0–34.3)	33.9 (33.4–34.4)	32.2–36.6
RDW (%)	15.1 ± 1.0	15.0 ± 0.8	13.2–17.4
Platelets ($\times 10^3/\mu L$)	185 ± 67	187 ± 76	177–398
Neutrophils (per μL)	4,600 (3,700–5,600)	4,700 (3,700–6,075)	3,100–14,400
Band neutrophils (per μL)	0 (0–0)	0 (0–0)	0
Lymphocytes (per μL)	1,100 (660–1600)*	1,200 (1,100–1,875)	900–5,500
Monocytes (per μL)	385 (210–530)	470 (250–697)	100–1,400
Eosinophils (per μL)	365 (210–540)*	510 (272–698)	0–1,600
Basophils (per μL)	0 (0–0)	0 (0–0)	0–200

Data are expressed as mean ± SD for normally distributed data and median (interquartile range) for non-normally distributed data.
*Significant ($P \leq 0.05$) difference between groups.
MCV = Mean corpuscular volume. MCH = Mean corpuscular hemoglobin. MCHC = Mean corpuscular hemoglobin concentration. RDW = Red cell distribution width.

Table 3—Results of serum biochemical analyses in SAR dogs that were deployed to 3 sites after the September 11, 2001, terrorist attacks and SAR dogs that were not deployed (control dogs).

Variable	Deployed dogs (n = 72)	Control dogs (52)	Reference range
Glucose (mg/dL)	96 ± 12*	85 ± 16	65–112
BUN (mg/dL)	16.6 ± 4.0	16.1 ± 3.8	9.0–33.0
Creatinine (mg/dL)	1.2 (1.1–1.4)	1.2 (1.1–1.3)	0.7–1.8
BUN:creatinine	13.6 (11.2–16.3)	13.5 (11.8–15.2)	NR
Phosphorus (mg/dL)	4.1 ± 0.6*	4.4 ± 0.8	2.8–6.1
Calcium (mg/dL)	10.2 (9.9–10.6)	10.2 (9.8–10.4)	9.8–11.7
Sodium (mmol/L)	146 (145–149)	147 (145–148)	140–150
Potassium (mmol/L)	4.4 ± 0.3*	4.6 ± 0.4	3.9–4.9
Chloride (mmol/L)	117 (115–119)	118 (116–120)	109–120
Enzymatic CO_2 (mmol/L)	22 (20–23)	21 (19–23)	17–28
Total protein (g/dL)	6.3 ± 0.6*	6.1 ± 0.5	5.4–7.1
Albumin (g/dL)	3.0 (2.9–3.2)	3.1 (2.9–3.3)	2.5–3.7
Globulin (g/dL)	3.2 (2.9–3.4)*	2.9 (2.7–3.2)	NR
Albumin:globulin	1.0 (0.9–1.1)*	1.1 (1.0–1.2)	NR
ALT (U/L)	46 (36–67)	46 (37–57)	16–91
AST (U/L)	34 (28–39)	34 (31–40)	23–65
Alkaline phosphatase (U/L)	51 (35–78)*	43 (34–55)	24–174
GGT (U/L)	13 (11–15)	13 (11–15)	7–24
Total bilirubin (mg/dL)	0.3 (0.2–0.4)*	0.2 (0.1–0.3)	0.3–0.9
Cholesterol (mg/dL)	218.5 ± 58.5*	194.9 ± 41.1	128.0–317.0
Anion gap (mmol/L)	13 (11–14)	12 (11–14)	12–16
Calculated osmolality (mOsm/kg)	284 ± 6	283 ± 4	NR

NR = Not reported. ALT = Alanine aminotransferase. AST = Aspartate aminotransferase. GGT = Gamma glutamyl transaminase.
See Table 2 for remainder of key.

centration. There was no relationship between age and bilirubin concentration or serum alkaline phosphatase activity. Conversely, age was positively related to cholesterol concentration. Thoracic radiographs (3 views) were evaluated, and no clinically relevant pulmonary abnormalities were identified in either group.

The behavioral survey was completed by handlers of deployed (95 completed) and control dogs (55 completed). There were no significant differences between the 2 groups for any of the 11 main behavioral traits, although the control dogs obtained significantly less favorable scores on 3 of the miscellaneous (unvalidated) behaviors, including eating its own or other animals' feces ($P = 0.03$), being nervous or fearful of going up or down stairs ($P = 0.01$), and pulling excessively hard on leash ($P = 0.005$). To overcome possible confounding effects of differences in breed composition between the groups, the analysis was repeated for German Shepherd Dogs only (29 deployed and 22 control German Shepherd Dogs were included in the behavioral analysis); deployed German Shepherd Dogs were given significantly higher (more favorable) scores for trainability.

Despite rumors of numerous SAR dog deaths, only 1 dog was confirmed to have died during the search period. This Port Authority of New York dog was killed during the collapse of the WTC Tower Two.[3] During the enrollment period (October 2001 to June 2002), there was 1 death in the deployed group and no deaths in the control group. In addition, information was received regarding the death of 2 other deployed dogs that were not enrolled in the study.

Discussion

The major long-term consequences of disaster response in humans are psychological and physical sequelae. There are no prior reports of either in SAR dogs. Although it was hypothesized that more medical or behavioral problems would be detected in the deployed dogs, compared with the control dogs, there were no clinically important differences between the groups at this time. The deployed dogs were older and had more experience than the control dogs. Both groups were composed predominantly of German Shepherd Dogs and Labrador Retrievers. Almost all dogs in both groups were trained to find live victims. The younger, less experienced control dogs spent more time in formal training than the older, more experienced deployed dogs.

Dogs responding to the September 11 disasters arrived soon after the attacks. The dogs were exposed to potential hazards, which were present in highest concentrations in the first few days after the attacks at the Pentagon and WTC. The exposure at the landfill was less likely to have been influenced by the temporal proximity to September 11; the material was constantly being sorted, aerosolizing any hazards that might have otherwise settled at ground zero.

At this early point in the study, the biochemical and hematologic differences found between control and deployed dogs were not considered clinically important, although they may represent early markers of subtle effects of the search response. Although all values were within reference ranges, significantly higher serum globulin concentration and associated higher total protein concentration and lower albumin-to-globulin ratios may have been the result of increased exposure to antigens at the disaster sites in the deployed dogs. This finding is consistent with the lack of relationship between globulins and age or previous search experience; however, it is possible that multiple other measured or unmeasured factors contributed to this difference between groups. In addition, the higher bilirubin concentration and alkaline phosphatase activity in the deployed dogs is consistent with an increased antigenic load, toxin exposure, or other hepatic insult. The higher cholesterol concentration of the deployed dogs was significantly and positively related to age and therefore may be explained by the older age of the deployed dogs.

Stress can result in hyperglycemia, lymphopenia, and eosinopenia, but the hematologic profile typically includes neutrophilia, and monocytosis,[13] which were not detected in the deployed dogs. Both the eosinophil concentration and blood glucose concentration are dynamic values, and a single sample may be influenced by the immediate stress of a veterinary visit and may not be representative of a chronic stressed state.

The absence of pulmonary abnormalities on thoracic radiographs is consistent with the study findings of human responders that developed coughing within 24 hours of exposure at the WTC.[9] Thoracic radiography is an insensitive tool for the early diagnosis of pulmonary malignancies. During the first two thirds of the course of a neoplastic disorder, the tumor nodules are too small to be recognized radiographically.[14,15] Additionally, expect-

ed lung lesions from chronic irritation caused by inhaled particulate matter or development of neoplasia would be a delayed change.

In humans, it takes at least 20 years[16] to develop mesothelioma after chronic asbestos exposure. In a report[17] that associated asbestos and mesothelioma in dogs, the mean age of affected dogs was 8.0 ± 1.9 years. The shorter life span of dogs results in a relatively shorter latency for cancer development, compared with humans. Therefore, the deployed dogs may provide an early indication of asbestos exposure in all rescue workers. In addition, silicosis may be evident in the dogs prior to the humans. These conditions emphasize the potential for dogs to act as sentinels of human disease as they did in Vietnam.[10]

Although behavioral trait profiles were not different between groups, the control dogs had evidence of problematic behavior with respect to some of the unvalidated miscellaneous questionnaire items. These results, together with the higher trainability score of the deployed German Shepherd Dogs, suggest a slight initial sampling bias toward behaviorally superior dogs among the deployed group, rather than any improvement or change in behavior after deployment. However, because predeployment data on these dogs were not available, the latter possibility cannot be excluded at this stage.

In human rescue workers, severe symptoms of PTSD are experienced by as many as 1 of every 3 rescue workers.[18] The lack of baseline behavioral data on the dogs did not allow us to identify stress-induced behavioral changes, however subtle, such as fear or aggression that would suggest postdeployment effects. It is also recognized in humans that symptoms of PTSD are most commonly evident within 6 months[19] of the traumatic event, but occasionally may not be obvious for several years.[20] Therefore, changes in the behavioral profile may still provide valuable information about the effects of SAR work in general and, more specifically, in association with the September 11 response. Because of the strong human-animal bond between the handlers and dogs, it is also possible that PTSD of the handler may actually cause physical or behavioral problems in the dogs. As part of a related study, a team of psychologists is monitoring the enrolled canine handlers.

The ability to identify abnormalities in the deployed dogs was limited in several ways. First, there was no predeployment evaluation of the dogs. Therefore, subtle changes in behavior, hematologic values, serum biochemical values, or radiographs in individuals cannot be identified. The data reported here, however, will serve as a baseline to identify changes over time. Second, although 97 deployed dogs were enrolled, if the prevalence of disease was low, there would be insufficient power to identify an association. There was potential for unrecorded conditions, such as lack of food, environmental factors, and sample handling or other unanticipated physiologic factors, to contribute to the observed differences in blood results between groups; however, systematic bias attributable to any of these factors seems unlikely. The nature of the survey data also introduced error. Despite aggressive recruitment attempts, some handlers were not identified and surveys were not completed until 9 months after the attacks. Most responses were designed to be yes or no; however, numerical reports such as duration of work shifts and time rested were subject to great variability. Additionally, medical problems may have developed in some dogs after completion of the initial survey, and this information will not be available until the second-year survey is completed. In addition, the limited response rate may have introduced a bias into our results because dogs belonging to nonparticipating handlers may have different problems. For example, the offer of health insurance may have encouraged handlers of dogs with a medical problem to enroll; alternatively, handlers of dogs that developed problems may also have been so overwhelmed by the dog's medical problems that they did not feel able to participate. Additionally, less qualified handlers may have been reticent to document problems in their dogs, whereas full-time handlers (eg, police officers) were often too busy to complete the paperwork. It is impossible to predict the exact nature of the bias that this limited enrollment may have introduced. To limit this bias, however, the study team recorded and confirmed any known adverse event (obtained through the media, the SAR community, or the handler) in deployed dogs whether or not they were enrolled in the study.

In an attempt to provide a measure of the degree of training, certification information was requested. It is difficult to compare training because the standards for certification vary considerably. The FEMA certification standards are the most consistent standards published[21] for both the performance of the dog and the handler, but it is difficult to compare training among other non-FEMA dogs.

The evaluation of the SAR dogs' respiratory system was limited to radiographs, which are insensitive to functional changes; however, of 3 dogs in which cough was reported during deployment, only 1 dog (deployed at the landfill) was reported to have clinical signs compatible with persistent increased airway reactivity.[i] No information was available on the prevalence of cough in workers at the landfill. In human workers at ground zero, the likelihood of developing cough was related to exposure; the workers present at the time of collapse composed the highest risk group. The moderate risk group included responders present in the first 2 days after the collapse of the WTC; most dogs fit into this category. Additionally, like the dogs, 78% of the human responders did not regularly use respiratory protection. However, of the 10,116 responders evaluated, 3% of the 6,958 with moderate exposure developed a cough. It is possible that our limited sample size contributed to the absence of chronic cough in the study dogs.[9]

Although validated for companion dogs and designed to be completed by anyone reasonably familiar with a dog's typical behavior, the behavioral survey has not been validated specifically for SAR dogs. Temperament traits considered advantageous in SAR dogs, such as high energy and high drive, are not necessarily the same as those deemed suitable for either companionship or other working roles, and it is not yet

known whether the behavior of these dogs can be compared reliably with other canine populations. Also, although the survey covers a wide range of salient canine behaviors, it is possible that it fails to measure specific behaviors that are of critical importance to evaluating SAR dogs and their responses to stress.

This is the first large-scale study evaluating SAR dogs. These data can be used to establish reference values for the dogs, help to standardize training protocols, and provide a central database to monitor the health of these dogs. Tracking the dogs and keeping a record of possible risk factors from the time of deployment will help identify the medical hazards of SAR work and the associated risk factors. Importantly, these dogs may also serve as sentinels for human disease.

The lack of clear adverse medical or behavioral effects of the September 11 response is encouraging. It must be emphasized, however, that the time period required for development of neoplasia or other diseases may be substantial. Greater values for globulin concentration, bilirubin concentration, and alkaline phosphatase activity in the deployed dogs, compared with the control dogs, may represent evidence of increased exposure to antigens or toxins. It is unknown whether this exposure could lead to any adverse sequelae during the next several years. The acute exposure to concentrated airborne hazards and the intense nature of the search activity are both unprecedented. Continued vigilance is warranted for the benefit of the SAR dogs and the human responders.

[a]Alvarezn J, Hunt M, Moser R, et al. Psychological sequelae in canine search and rescue handlers after September 11th. Poster presented at the 36th Annual Meeting of the Association for the Advancement of Behavior Therapy, November 2002; Reno, Nev.

[b]Veterinary Pet Insurance Co, Brea, Calif.

[c]Cell Dyne 3500, Abbott Diagnostics, Santa Clara, Calif.

[d]Vitros System Chemistry 250, Johnson & Johnson Clinical Diagnostics, Rochester, NY.

[e]Copies of the medical and behavioral surveys are available from the corresponding author.

[f]Canine Behavioral Assessment & Research Questionnaire (CBARQ), James A. Serpell, PhD, University of Pennsylvania School of Veterinary Medicine, Philadelphia, Pa.

[g]SigmaStat for Windows, version 2.03, SPSS Inc, Chicago, Ill.

[h]StatView, version 5.0.1, SAS Institute Inc, Cary, NC.

[i]Shearer T, Shearer Pet Hospital, Columbus, Ohio: Personal communication, 2001.

References

1. US Department of State. Office of International Information Programs. September 11 one year later. A selected chronology of key events, September 11, 2001—present. Available at: usinfo.state.gov/journals/itgic/0902/ijge/gjchron.htm. Accessed Jan 6, 2003.

2. CDC. Injuries and illnesses among New York City Fire Department rescue workers after responding to the World Trade Center attacks. *MMWR CDC Surveill Summ* 2002;51:1–5.

3. Remembering Sirius: a K-9 memorial service. Available at: our.homewithgod.com/mkcathy/sirius2.html. Accessed May 2, 2002.

4. Environmental Protection Agency. National Center for Environmental Assessment page. Exposure and human health evaluation of airborne pollution from the World Trade Center disaster (external review draft). Available at: cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=54667. Accessed Jan 6, 2003.

5. City of New York. Fresh Kills: landfill to landscape. About Fresh Kills. Available at: www.nyc.gov/html/dcp/html/fkl/ada/about/1_0.html. Accessed Jan 6, 2003.

6. Federal Emergency Management Agency. FEMA news page. World Trade Center and Pentagon disaster update. Available at: www.fema.gov/nwz01/nwz01_134.shtm. Accessed Apr 22, 2003.

7. Environmental Protection Agency. EPA response to September 11 page. Environmental Protection Agency Region III. October 9, 2001: summary of environmental monitoring operations at the Pentagon. Available at: www.epa.gov/wtc/pentagon-air-sampling.htm. Accessed Jan 6, 2003.

8. Duhaime RA, Norden D, Corso B, et al. Injuries and illnesses in working dogs used during the disaster response after the bombing in Oklahoma City. *J Am Vet Med Assoc* 1998;212:1202–1207.

9. Prezant DJ, Weiden M, Banauch GI, et al. Cough and bronchial responsiveness in firefighters at the World Trade Center site. *N Engl J Med* 2002;347:806–815.

10. Hayes HM, Tarone RE, Casey HW, et al. Excess of seminomas observed in Vietnam service U.S. military working dogs. *J Natl Cancer Inst* 1990;82:1042–1046.

11. Hsu Y, Serpell JA. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. *J Am Vet Med Assoc* 2003;223:1293–1300.

12. *SAS/STAT user's guide: version 8 edition*. Cary, NC: SAS Institute Inc, 1999;3884.

13. Schultze EA. Interpretation of canine leukocyte responses. In: Feldman BF, Zinkl JG, Jain NC, eds. *Schalm's veterinary hematology*. 5th ed. Philadelphia: Lippincott Williams & Wilkins, 2000;366–381.

14. Theilen GH, Madewell BR. Tumors of the respiratory tract and thorax. In: Theilen GH, Madewell BR, eds. *Veterinary cancer medicine*. Philadelphia: Lea & Febiger, 1979;341–346.

15. Suter PF, Lord PF. *Thoracic radiography: a text atlas of thoracic diseases of the dog and cat*. Wettswil, Switzerland: 1984;658–664.

16. Selikoff IJ, Hammond EC, Seidman H. Latency of asbestos disease among insulation workers in the United States and Canada. *Cancer* 1980;46:2736–2740.

17. Glickman LT, Domanski LM, Maguire TG, et al. Mesothelioma in pet dogs associated with exposure of their owners to asbestos. *Environ Res* 1983;32:305–313.

18. National Center for Post-Traumatic Stress Disorder. Disaster rescue and response workers: a National Center for PTSD fact sheet. Available at: www.ncptsd.org/facts/disasters/fs_rescue_workers.html. Accessed Mar 13, 2003.

19. Tomb DA. The phenomenology of post-traumatic stress disorder. *Psychiatr Clin North Am* 1994;17:237–250.

20. National Institute of Mental Health. When does PTSD first occur? Facts about Post-Traumatic Stress Disorder. Available at: www.nimh.nih.gov/anxiety/ptsdfacts.cfm. Accessed Mar 13, 2003.

21. Federal Emergency Management Agency. Urban Search and Rescue page. Canine readiness evaluation process. Available at: www.fema.gov/usr/canine.shtm. Accessed Feb 26, 2003.

Deployment morbidity among search-and-rescue dogs used after the September 11, 2001, terrorist attacks

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Objectives—To determine characteristics, variables associated with deployment morbidity, and injuries and illnesses of search-and-rescue dogs associated with the Sept 11, 2001, terrorist attacks.

Design—Historical cohort study.

Animals—96 dogs.

Procedure—Data collected included previous medical or surgical history, physical attributes of dogs, type and number of years of training, site of deployment, shift and hours worked, and number of days deployed. Combined morbidity was defined as 1 or more abnormalities of body systems, including traumatic injuries.

Results—Handlers of 96 of the 212 dogs responded to the surveys. Fifty-nine dogs were deployed by the Federal Emergency Management Agency, 10 by police forces, and 27 as members of other search-and-rescue teams. Sixty-five dogs (incidence rate, 17 events/1,000 dog search hours) had combined morbidity during deployment. System-specific morbidity rates included gastrointestinal tract signs (5 events/1,000 dog search hours), cuts and abrasions mostly on the feet (5 events/1,000 dog search hours), fatigue (6 events/1,000 dog search hours), change in appetite (6 events/1,000 dogs search hours), dehydration (5 events/1,000 dog search hours), respiratory tract problems (2 events/1,000 dog search hours), heat exhaustion (2 events/1,000 dog search hours), and orthopedic or back problems (2 events/1,000 dog search hours). Dogs deployed to the World Trade Center were 6.6 times more likely to have combined morbidity, compared with dogs at the Pentagon.

Conclusions and Clinical Relevance—Injury and illnesses occurred in most dogs and affected several organ systems, but all were minor. (*J Am Vet Med Assoc* 2004;225:868–873)

The events of September 11, 2001, constituted the worst terrorist attack on American soil in history. A portion of the Pentagon was reduced to rubble, killing 189 people. An additional 2,829 lives were lost as a result of the collapse of the **World Trade Center (WTC)**,[1] an area of 16 acres. Approximately 11,000 firefighters and emergency medical personnel responded to the attacks.[2] Among the responders were an estimated 250 to 300 **search-and-rescue (SAR)** dogs and their handlers. These dogs were involved in SAR efforts at the WTC and Pentagon and search and recovery efforts at the Fresh Kills Landfill on Staten Island. The **Federal Emergency Management Agency (FEMA)** deployed 80 dogs as members of 20 certified urban SAR teams to support the rescue operation in New York. Five other FEMA teams responded to the Pentagon site. Police dogs and other organized SAR teams were also instrumental in the disaster response.

The Oklahoma City bombing disaster was the only occasion for similar use of dogs for SAR efforts. In the aftermath of the bombing, an epidemiologic study was conducted to determine the medical problems among the responding SAR dogs.[3] Because no man-made national disaster of the magnitude seen on September 11, 2001, has been described, information regarding the medical effects of such a disaster on dogs involved in the response is largely unknown. The purposes of the study reported here were to describe characteristics of the responding dogs, evaluate variables associated with morbidity of the dogs, describe canine injuries and illnesses that occurred during deployment, and offer recommendations to decrease morbidity in future response efforts.

Materials and Methods

Attempts were made to contact all dog handlers (identified by use of a list generated by the American Kennel Club) who participated in the rescue efforts of the WTC and Pentagon and in the recovery efforts at the landfill. The list included FEMA-deployed dogs and dog-handler teams who self-deployed or were members of other organized SAR teams. Additional individuals contacted the research group after hearing of the study through the media or other handlers. Of the 212 teams contacted, 96 (45%) completed a predeployment health survey and a deployment health survey. Only completed responses were included, and a signed consent form accompanied all completed surveys. Incentive for participation in the study included a health insurance policy for each dog.[a] Although present at the WTC, those dogs deployed primarily for therapy and stress relief of the rescue workers were not included in this study.

Data collection began in October 2001 and ended June 2002. Predeployment surveys included questions regarding medical and surgical history, age, sex, breed, diet, and body weight. Deployment surveys included questions regarding deployment dates, site of deployment, shift and hours worked, and medical or surgical problems encountered during deployment. Additional information requested included state of origin for each dog-handler team, prior training, type of training, level of training, and years of active search. Morbidity was defined as 1 or more injuries or illnesses, including respiratory tract disorders, dehydration, weight loss, change in appetite, urinary tract problems, vomiting, diarrhea, cuts or abrasions, lameness or back problems, skin

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disorders, ocular or aural problems, and decreased physical stamina (tired easily, excessively tired, or heat exhaustion). Variables evaluated for association with morbidity included age, sex, breed, weight, medical or surgical history, deployment site, total hours spent in search, handler's medical problems during deployment, shift worked during deployment, years of training, years of active search, type of training, FEMA certification, and whether or not canine search was a full-time job.

Statistical analyses—Continuous variables were assessed for normality by visual inspection and the Shapiro-Wilks test. Mean ± SD or median (range) values were used to describe parametric or nonparametric continuous variables, respectively. Incidence rates were calculated from number of events that occurred divided by the number of dog hours spent in active search. Number of dog hours spent in active search was determined from the number of shifts worked by each dog and the mean number of hours worked per shift by each dog. All incidence rates are reported as number of events per 1,000 dog search hours. Poisson exact 95% confidence intervals for each incidence rate were calculated.

For comparing continuous variables other than incidence rates, the Wilcoxon rank sum test or the unpaired *t* test for equal variances was used, depending on data distribution. The Fisher exact test or Pearson χ^2 test was used where appropriate when comparing 2 proportions.

Logistic regression was used for the multivariate analysis. The outcome variable was morbidity (yes or no). A dog was considered to have a yes result for morbidity if it had any of the individual morbidities that were evaluated in the survey. Morbidity was considered a no result if the dog had none of the individual morbidities that were evaluated in the survey. Because of the limited number of outcomes, the most plausible independent variables (determined by the authors) that might be associated with outcome were included for initial model development. These independent variables included site, type of training, previous medical or surgical problems, FEMA certification status, sex, age, whether they had a live or cadaver find, and hours spent in active search. Hours worked and variables that had a coefficient that had an associated P value < 0.2 from this initial full model were entered into a second model. The final model was developed by retaining hours worked and eliminating any variables with coefficients that had $P \geq 0.05$. The fit of the resulting model was tested by use of the Hosmer-Lemeshow goodness-of-fit χ^2 statistic. All statistical evaluations were performed with a statistical software program.[b]

Results

Ninety handlers involved in the SAR or search and recovery efforts of September 11, 2001, completed surveys on 96 deployed dogs. The combined number of hours in active search was 3,709. Sixty-one (63.5%) of these dogs were deployed to the WTC, 23 (24%) to the Pentagon, and 12 (12.5%) to the landfill.

Signalment—Median age of the deployed dogs was 5 years (range, 1 to 11 years). The most common breeds deployed were German Shepherds (n = 31), Labrador Retrievers (n = 28), and Golden Retrievers (n = 12). Other breeds included mixed breeds (n = 8); Border Collies (n = 7); Australian Shepherds (n = 4) and 1 each of Belgian Tervuren, Doberman Pinscher, English Springer Spaniel, Giant Schnauzer, Beauceron, and Rottweiler. Dogs weighed 31 ± 7 kg (68 ± 15 lb). Fifty-four (56%) dogs were male (18 sexually intact and 36 castrated) and 41 dogs (43%) were female (2 sexually intact and 39 spayed); for 1 dog, the sex was not reported. Dogs had a median of 4 years' search experience (range, 0 to 12 years) and had a combined 486 years of training (median, 5 years; range, 1 to 12 years). Fifty (52%) dogs were deployed for the first time during this disaster.

Dogs and distribution among sites—Twenty-three dogs representing 4 states were deployed to the Pentagon, where they spent a median of 12 days (range, 1 to 13 days). The landfill site had 12 dogs deployed from 6 states for a median of 7 days (range, 1 to 12 days). The first canine-handler teams were on site at the landfill on September 17, 2001, 5 days after the first debris from the WTC arrived. The WTC site included 61 dogs from 16 states, deployed for a median of 10 days (range, 1 to 25 days).

Training and experience of dogs—Fifty (52%) deployed dogs were certified as urban SAR dogs by FEMA. These dogs were equally distributed between those with basic and advanced certification. An additional 9 (9%) dogs were affiliated with FEMA, but not certified. Those dogs trained to FEMA standards and were deployed prior to certification because of their recognized need during the rescue efforts. Police dogs (n = 10 [10%]) and members of other organized canine SAR teams (dogs and handlers) (n = 27 dogs [28%]) represented the non-FEMA certified responders. For 20 canine-handler teams, SAR was reported as a full-time occupation. Ninety-two (96%) of the dogs were trained to find live victims (live find), and 58 (60%) dogs were trained to find human remains (cadaver find). Of these dogs, 54 (56%) were trained for both live and cadaver finds (dual trained). Additionally, 16 (16.7%) dogs were solely trained for urban SAR, whereas 13 (13.5%) dogs were trained only for wilderness SAR. Sixty-seven (70%) dogs were trained for both urban and wilderness SAR.

Cadaver and live finds—Handlers reported that seventy (73%) dogs had positive cadaver finds and 4 (4%) dogs alerted for live finds. Live alerts (actions of the dog that indicate a find) were reported from the WTC and may have represented alerts on other rescue workers, because no live-victim finds by SAR dogs could be confirmed. Of the 70 teams reporting cadaver finds, 41 (58%) were located at the WTC, 18 (26%) were at the Pentagon, and 11 (16%) were at the landfill. Sixteen (18%) handlers did not report any cadaver finds, and 10 (9%) did not respond to the question. In general, the dogs signaled a live find with a bark alert. A more passive alert (sitting or scratching at the ground) signified a cadaver find. For all dogs, play (n = 85), food (22), or praise (12) was the reward for finds. Because many dogs were trained in live find and rewarded with play, efforts were made, when possible, to stage live finds in areas away from the actual work area to reinforce their training and reward their find.

Previous medical conditions—Previous medical conditions were described in 46 (48%) dogs. Among the most common complaints were musculoskeletal disorders (n = 21), atopy (11), and borreliosis (7). Only 2 dogs with previously described musculoskeletal

problems had recurrence of lameness while deployed. Twenty-nine (30%) dogs had 33 surgical procedures before deployment. Of those surgeries, 10 were orthopedic procedures and 23 were soft tissue surgeries (spays and castrations were not included).

Morbidity—Sixty-five (68%) dogs had morbidity during deployment for an incidence rate of 17 events/1,000 dog search hours (95% CI, 13 events/1,000 hours to 17 events/1,000 hours). System specific incidence rates ranged from 1 event/1,000 hours to 9 events/1,000 hours, and the overall incidence of morbidity at each site was also determined (Table 1). Thirty-four (35%) dogs had cuts or abrasions, including superficial cuts (n = 20), superficial abrasions (3), full-thickness wounds (6), punctures (5), or laceration (1), which comprised the highest morbidity incidence rate during deployment. Only 1 dog required sutures (for a laceration above the metacarpal pad); this was the only surgical procedure during deployment. Most wounds were located on the feet, footpads, or limbs (24/34 [70%]). Four of 9 dogs that wore foot protection had cuts and abrasions, 3 had injuries to the feet or pads, and 1 had a superficial cut on the mouth. No dogs deployed to the Pentagon wore foot protection. There was no significant difference in incidence of cuts and abrasions among sites. There was a higher incidence (not significant) of wounds in dogs that did not wear foot protection (10.2 events/1,000 hours; 95% CI, 6.8 events/1,000 hours to 14.0 events/1,000 hours) versus those that did wear foot protection (4.7 events/1,000 hours; 95% CI, 1.3 events/1,000 hours to 11.9 events/1,000 hours). Additionally, the overall incidence of wounds was most similar between the WTC (11.2 events/1,000 hours; 95% CI, 7.4 events/1,000 hours to 16.3 events/1,000 hours) and the landfill (8.9 events/1,000 hours; 95% CI, 2.4 events/1,000 hours to 22.7 events/1,000 hours). Other skin disorders reported were pruritus or erythema (n = 11), hair loss (2), and undescribed (1).

Twenty-one of the 96 (22%) dogs had gastrointestinal tract signs while deployed, including vomiting and diarrhea. Sixteen (17%) dogs had diarrhea and 5 (5%) dogs accounted for 6 episodes of vomiting. Weight loss was documented in 22 (23%) dogs during deployment and was the second most common problem, with an overall incidence rate of 6.3/1,000 hours. Dogs that lost weight weighed significantly ($P = 0.003$) more (median weight, 33 kg [73 lb]; range, 25 to 51 kg [55 to 112 lb]), compared with dogs that did not lose weight (median weight, 30 kg [66 lb]; range, 17 to 50 kg [37 to 110 lb]), and most dogs with weight loss had a concurrent decrease in appetite. Of the 22 (23%) dogs with a reported change in appetite, only 3 dogs had an increased appetite.

Eighteen of 19 dogs with dehydration were treated with 38 doses of fluids administered SC (n = 16) or IV (2). Dehydration was not related to total number of hours searching through the rubble, shift length, shift worked (day, night, or both), or the number of days deployed. Incidence rates of vomiting, weight loss, and change in appetite were greater in dogs that were dehydrated (3 events/1,000 hours [95% CI, 0.6 to 8.6 events/1,000 hours]; 7.8 events/1,000 hours [95% CI, 3.4 to 15.5 events/1,000 hours]; and 12 events/1,000 hours [95% CI, 6 to 20 events/1,000 hours], respectively) versus those that were not dehydrated (0.4 events/1,000 hours [95% CI, 0.01 to 2 events/1,000 hours]; 5.3 events/1,000 hours [95% CI, 2.8 to 9.1 events/1,000 hours]; and 3.7 events/1,000 hours [95% CI, 1.6 to 6.9 events/1,000 hours], respectively), although differences were not significant. The incidence of dehydration was significantly greater in dogs deployed to the WTC (7.6 events/1,000 hours; 95% CI, 4.5 to 12 events/1,000 hours) than dogs deployed to the Pentagon (0.0 events/1,000 hours; 1-sided 97.5% CI, 0.0 to 4.3 events/1,000 hours), but there was no significant difference between the WTC and the landfill (2.2 events/1,000 hours; 95% CI, 0.06 to 12.4 events/1,000 hours).

Table 1—Incidence rates (No. of morbidities/1,000 dog hours of searching) for various morbidities in search-and-rescue dogs at the World Trade Center (WTC), the Pentagon, or a landfill for debris from the WTC collapse of September 11, 2001.

Morbidity	Site	Incidence rate/1,000 dog search hours	95% CI
Cuts/abrasions/ punctures	WTC	11.2	7.4–16.3
	Pentagon	3.5	0.7–1.0
	Landfill	8.9	2.4–22.7
	Overall	9.2	6.4–12.8
Weight loss	WTC	7.8	4.6–12.4
	Pentagon	3.7	0.8–10.8
	Landfill	2.5	0.06–14.1
	Overall	6.3	3.9–9.5
Dehydration	WTC	7.6	4.5–12.0
	Pentagon	0.0	0.0–4.3*
	Landfill	2.2	0.06–12.4
	Overall	5.2	3.1–8.1
Change in appetite	WTC	7.5	4.4–11.8
	Pentagon	1.2	0.03–6.6
	Landfill	6.6	1.4–19.4
	Overall	5.9	3.7–8.9
Tires easily/ excessively tired	WTC	5.8	3.2–9.9
	Pentagon	4.7	1.2–12.1
	Landfill	7.6	1.6–22.2
	Overall	5.7	3.6–8.8
Gastrointestinal problems	WTC	5.8	3.2–9.8
	Pentagon	3.5	0.7–10.3
	Landfill	6.9	1.4–20.2
	Overall	5.4	3.3–8.4
Orthopedic problems	WTC	3.3	1.4–6.5
	Pentagon	0.0	0.0–4.3*
	Landfill	2.2	0.06–12.4
	Overall	2.4	1.1–4.6
Heat exhaustion	WTC	2.1	0.7–4.8
	Pentagon	0.0	0.0–4.3*
	Landfill	2.2	0.06–12.4
	Overall	1.6	0.6–3.5
Urinary tract problems	WTC	2.1	0.7–4.8
	Pentagon	0.0	0.0–4.3*
	Landfill	0.0	0.0–8.2
	Overall	1.3	0.4–3.1
Respiratory tract problems	WTC	2.0	0.7–4.8
	Pentagon	1.2	0.03–6.5
	Landfill	4.4	0.5–16.0
	Overall	2.2	0.9–4.2

*One-sided 97.5% confidence interval (CI).

Twenty-three (24%) dogs had fatigue. Handlers reported that their dogs had 28 episodes of fatigue (either excessively tired or tired easily), with an incidence rate of 5.7/1,000 hours. Six dogs were described as having heat exhaustion. Median time spent in search for the dogs that had heat exhaustion was 11 hours (range, 1.5 to 60 hours), compared with dogs that did not have heat exhaustion (median, 29 hours; range, 0.75 to 264 hours). Although clinically important, this difference was not significant. Three of the 6 dogs with heat exhaustion worked for only 1 day. One of the dogs was FEMA-trained and certified; this dog remained at the WTC for 14 days. Five of 6 dogs with heat exhaustion were deployed to the WTC.

Eight (8%) dogs were reported to have lameness described as a slight limp with activity (n = 4), a noticeable limp with rest (3), and a noticeable limp with activity (1). Three of these dogs were treated with carprofen[c] for pain management. Three of 4 dogs with reported back problems had lameness in both hind legs. One of these dogs had possible intervertebral disk disease immediately prior to deployment.

Eight percent of the dogs in this study had respiratory disorders during deployment. Five of the 8 dogs with respiratory problems were deployed to the WTC, although the highest incidence rate was at the landfill (4 events/1,000 hours; 95% CI, 0.5 to 16 events/1,000 hours). Of the dogs at the WTC, 2 dogs had an increase in respiratory effort, whereas the remaining 3 had increases in respiratory rate in addition to coughing and sneezing. All of the dogs deployed to the WTC that developed respiratory problems were deployed in the first 2 days of operation. Two of those dogs were deployed for only 1 day. Median search hours spent for the dogs that had breathing problems was 17 (range, 3.5 to 264 hours), compared with dogs that did not have breathing problems (median, 26 hours; range, 0.75 to 238); although clinically important, this difference was not significant. No handler who completed the survey information had a dog that required oxygen therapy. However, 1 dog whose handler did not fully complete the survey information and was not included in the analysis was known to have aspirated large amounts of dust at the WTC and required evacuation and oxygen therapy. This dog was treated on site by 1 of the authors (CMO).

Five (5%) dogs had urinary tract problems, including hematuria (n = 2), straining to urinate (1), polyuria (1), and infection (1). Ocular problems occurred in 8 (8%) dogs and included redness (n = 4), mild discharge (4), and squinting (3). Of the 8 dogs with ocular problems, 4 had bilateral involvement.

Medications were given to 37 of 96 (38.5%) dogs during deployment. The most commonly reported medications were antimicrobials for skin lesions (n = 11), metronidazole[d] for gastrointestinal tract signs (6), and carprofen[c] for musculoskeletal disorders (5). Two dogs received levothyroxine sodium[e] for previously diagnosed hypothyroidism, and 3 others received glucosamine-chondroitin for previously diagnosed joint disease.

Although not significant, it was interesting to note that dogs belonging to handlers who had medical problems had a higher incidence of medical problems (26.3 events/1,000 hours; CI, 15.6 to 41.6 events/1,000 hours) than dogs of handlers who did not have medical problems (15.3 events/1,000 hours; CI, 11.1 to 20.6 events/1,000 hours). Additionally, handlers at the WTC (21/57 [37%]) were found to have more medical problems than those deployed to the Pentagon (2/22 [9%]; $P = 0.002$). This could not be adjusted for total search hours because some handlers worked with more than 1 dog during deployment.

Multivariate analysis—Nearly all variables dropped out of the initial full model. Remaining variables for the second model included training for live finds and site. Training for live finds was eliminated from the second model ($P = 0.117$), which left total hours and site as the remaining variables in the final model. The final model was based on 90 observations. The overall χ^2 of the model was 13.31 (3 *df*) and was significant ($P = 0.004$). The *P* value for the Hosmer-Lemeshow goodness of fit was 0.2397, which indicated an adequate fit to the data.

Dogs were 6.6 times (95% CI, 2.2 to 20 times) as likely to have morbidity at the WTC, compared with the Pentagon, when controlling for other sites and total dog hours of active search. There were no significant differences among any of the other sites. Total hours were not significantly associated with morbidity (OR, 1.007; 95% CI, 0.99 to 1.02).

Discussion

Sixty-five dogs had morbidity during deployment to the WTC, the Pentagon, or the landfill. Given the mass destruction and intensity of the working environment, it is surprising that most injuries and illnesses were minor and did not detract from the SAR or recovery operations. Those dogs deployed to the WTC had significantly more injuries than those at the Pentagon. Deployment to this site was the only variable significantly associated with outcome. This site-dependent effect was considered plausible because the collapse of the towers and adjacent structures was dissimilar to the damage at the Pentagon and resulted in an enormous disaster zone. The initial respondents were exposed to a variety of particulate matter that was airborne as a result of the strength of the towers' collapse, in addition to explosions, fire, and falling debris.[2]

Although the overall incidence rates for individual morbidities were low, the potential clinical implications of the described morbidities were important. Some of the reported illnesses did lead to early departure from the sites for the canine-handler teams. These illnesses included heat exhaustion and respiratory difficulty. Although these illnesses were reported infrequently among handlers who responded to the surveys, it appears that they occurred early in the deployment period and some dogs did not return to searching as a result. The respiratory difficulty seen in this population of dogs was much less severe than has been described in humans in the immediate aftermath of the collapse and the 12-month period after the collapse. During the 48 hours after the attacks, approximately 90% of the rescue workers reported an acute cough,

often accompanied by nasal congestion and tightness or a burning sensation in the chest.[2] Of rescue workers at the WTC at the time of collapse, 8% had a disabling persistent cough associated with decreased lung function and 23% had bronchial hyperresponsiveness.[4] Within the first 2 days after the collapse, 3% of the workers had the same type of cough and 8% had bronchial hyperresponsiveness.[5] Respirators were worn by only 7% of the firefighters on the day of the collapse and increased in use to 65% by the second week.[5] Because none of the dogs wore any respiratory protection, a higher incidence of reported respiratory difficulty than was described was anticipated, given the identical working conditions. However, none of these dogs were present during the collapse of the towers, when the concentration of airborne particulate matter was highest.

Fatigue was commonly described in this population of search dogs. Most dogs had periods of tiring easily or becoming excessively tired. Only a small percentage of dogs had heat exhaustion. Few dogs worked < 60 minutes before resting during their shift, and many dogs worked > 8 to 12 hours. This long work schedule prevented adequate rest time for the dogs and the handlers. The FEMA recommendations include a shift length of 12 hours, and for every 20 to 45 minutes of work, rest is recommended for an equal period of time.[6] From the survey data, it is clear that few handlers, either FEMA or non-FEMA, followed a set shift length or rest schedule. Given the intensity of the environment at the disaster sites and the recognized need for dogs in the recovery efforts, they may have worked for longer periods of time without adequate rest. However, strict shift and rest schedules should be enforced to allow needed recovery time, improved search efficiency, and safety. Results of a recent study[7] support this recommendation because dogs become less efficient and accurate in their ability to detect scent after strenuous physical activity.

Weight loss was reported with a frequency second only to cuts and abrasions. Most dogs with weight loss had decreased appetite. The extended shifts worked without adequate periods of rest as well as the intensity and stress of the working environments likely led to decreased food and water intake, undoubtedly contributing to dehydration in some dogs. Additionally, dogs may require a more nutrient-dense food to meet the added energy requirements of work. This is supported by the fact that larger dogs (ie, dogs that weighed more) were more likely to lose weight throughout deployment than smaller dogs (ie, dogs that weighed less). Mainly on the basis of results of studies[8] performed in racing Greyhounds and sled dogs, energy intake should be increased by 40% to 50% for 1 day of work. This energy most often should be supplied as protein, rather than carbohydrates.[8] Relative to body size, dogs metabolize free fatty acids at a higher rate humans do. Dog muscle is therefore more adapted to use fat than human muscle is,[9] making the ideal diet for endurance work a high-protein, high-fat, low-carbohydrate diet. Endurance dogs may benefit from carbohydrate supplementation immediately after exercise to help maintain adequate muscle glycogen stores.[10] Although most of the dogs involved in SAR are not typically considered endurance athletes, they are exposed to similar stresses, both organic and behavioral, that affect their state of nutrition.[8] It may be possible therefore to extrapolate the results obtained from sled dog nutrition studies[9] to SAR dogs.

Few dogs had foot protection during deployment. Despite this finding, serious wounds were uncommon, with surgery required in only 1 dog. However, superficial cuts and abrasions were common and had the highest incidence rate of any morbidity. Most of the cuts and abrasions were on the feet or footpads of the dogs, and there was a higher incidence (although not significant) of wounds in those dogs that did not have foot protection, indicating that foot protection may be beneficial in reducing the incidence of injuries. Concerns voiced by handlers and FEMA regarding this issue are similar to those reported in the epidemiologic study[3] of the Oklahoma City bombing disaster site. According to the FEMA Web site dedicated to the events of September 11, 2001, dogs often need to perform a soft walk and splay their paws for maximum traction. In addition, booties can sometimes add to the risk of searching in tight or obstructed spaces.[6] Perhaps a type of foot protection can be developed that will combine needed traction and workability with desirable safety.

The similarities between the WTC and landfill sites may help explain the high incidence of superficial wounds among dogs deployed to these sites. A large amount of debris, mostly scrap metal, from the WTC was searched at the landfill. As such, the dogs were exposed to similar hazards and had similar wounds.

Recommendations made here are based on the descriptive information provided in this report. Many of these recommendations are similar to those made as a result of the Oklahoma City bombing.[3] The number of dogs at the disaster sites could only be estimated. There was no central station for registering dogs and handlers, which made it possible for people to self-engage in the disaster response. Lack of verification of experience and training likely put those dogs and handlers at increased risk. A central registration facility should be established to ensure that all known participants in the disaster response are appropriately trained and accounted for. A master schedule should be devised and enforced to provide adequate shift coverage and enforce important rest periods. There should also be a central treatment facility, similar to what was provided by Suffolk County, the Long Island Veterinary Medical Association, and Veterinary Medical Assistance Teams,[11] to address the medical needs of the dogs. Such a facility would allow adequate records to be generated on each dog treated so that injuries and illnesses can be recognized and appropriate follow-up care could be provided as needed.

Animal care needs to be integrated into the disaster response. Although most of the injuries and illnesses reported here were minor and likely did not detract from the search, the number of morbidities emphasizes the need for veterinary care in SAR dogs. Veterinarians should be members of SAR teams or, alternatively, these teams should have preexisting relationships with

the Veterinary Medical Assistance Teams to provide their dogs with proper medical care during a disaster response. Local veterinary resources should also be identified, incorporated, and used, particularly for any extended, surgical, or intensive care.

An off-site respite area should be designated for the dogs and their handlers to use between shifts. This space would serve as a rest area for the dogs. It was undoubtedly difficult for the dogs to eat, drink, and sleep among the activity of the rescue operations, and this was reflected in some of the reported morbidities. The rest site should be situated away from other rescue workers to help keep stress and activity to a minimum. A set sleep-wake schedule (shift schedule) should be developed to encourage longer periods of sleep and less disruption to sleep-wake cycles, which is beneficial in urban dogs engaging in shift work.[12]

This study investigated a limited population of dogs and handlers. Handlers may have been more likely to respond to the surveys if they were deployed as members of organized SAR groups and, as such, considered legitimately deployed. Those individuals were more easily identified and contacted than those who self-deployed. In addition, handlers who were not qualified may have been reluctant to participate in this study. Our population may also have been influenced by the health insurance incentive offered for participation in the study. Handlers whose dogs had medical problems during deployment may have been more likely to respond, given this incentive. If our interpretation is correct, we may have overestimated the number of dogs that developed morbidity during deployment. Nonetheless, our limited sample size may have restricted our ability to detect significance with regards to individual morbidities and, therefore, underestimated the importance of the described injuries and illnesses.

The information was provided in survey form and, as such, allowed for interpretation of questions and answers. Some of the surveys were not completed until almost 1 year after the tragic events of that day. It is possible that some information would have been represented or remembered differently immediately after the attack. However, the information was valuable in creating profiles of the deployed dogs and making recommendations for their use in future disasters.

[a]Veterinary Pet Insurance Co, Brea, Calif.
[b]Intercooled Stata for Windows, version 7.0, College Station, Tex.
[c]Rimadyl, Pfizer Inc, Exton, Pa.
[d]Flagyl, Sidmak Laboratories Inc, East Hanover, NJ.
[e]Soloxine, Daniels Pharmaceuticals, St Petersburg, Fla.

References

1. US Department of State. Office of International Information Programs. September 11 one year later. A selected chronology of key events, September 11, 2001—present. Available at: usinfo.state.gov/journals/itgic/0902/ijge/gjchron.htm. Accessed Jan 6, 2003.
2. CDC. Injuries and illnesses among New York City Fire Department rescue workers after responding to the World Trade Center attacks. *MMWR CDC Surveill Summ* 2002;51:1–5.
3. Duhaime RA, Norden D, Corso B, et al. Injuries and illnesses in working dogs used during the disaster response after the bombing in Oklahoma City. *J Am Vet Med Assoc* 1998;212:1202–1207.
4. Scanlon PD. World Trade Center cough—a lingering legacy and a cautionary tale. *N Engl J Med* 2002;347:840–842.
5. Prezant DJ, Weiden M, Banauch GI, et al. Cough and bronchial responsiveness in firefighters at the World Trade Center site. *N Engl J Med* 2002;347:806–815.
6. Federal Emergency Management Agency. News media page. Canine search and rescue teams' response to the 9/11 attacks. Available at: www.fema.gov/about/mediacanine.shtm. Mar 6, 2003.
7. Gazit I, Terkel J. Explosives detection by sniffer dogs following strenuous physical activity. *Appl Anim Behav Sci* 2003;81:149–161.
8. Grandjean D, Paragon BM. Nutrition of racing and working dogs. Part II. Determination of energy requirements and the nutritional impact of stress. *Compend Contin Educ Pract Vet* 1993;15:45–57.
9. Hill RC. Nutritional requirements of exercising dogs. *J Nutr* 1998;128:2686S–2690S.
10. Reynolds AJ, Carey DP, Reinhart GA, et al. Effect of postexercise carbohydrate supplementation on muscle glycogen repletion in trained sled dogs. *Am J Vet Res* 1997;58:1252–1256.
11. Otto CM, Franz MA, Kellogg B, et al. Field treatment of search dogs: lessons learned from the World Trade Center disaster. *J Vet Emerg Crit Care* 2002;12:33–42.
12. Adams GJ, Johnson KG. Sleep, work, and the effects of shift work in drug detector dogs *Canis familiaris*. *Appl Anim Behav Sci* 1994;41:115–126.

Medical Surveillance of Search Dogs Deployed to the World Trade Center and Pentagon: 2001–2006

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Abstract **In response to the terrorist attacks of September 11, 2001, at the World Trade Center and Pentagon, almost 50,000 rescue workers and approximately 300 search and rescue dogs participated in rescue and recovery operations. The dogs were exposed to the same hazards as the human workers, but did not have any of the personal protective gear. This prospective double cohort observational study compared annual medical history, blood biochemical and hematologic results, and thoracic radiographic findings in 95 search and rescue dogs that responded to the terrorist attacks at the World Trade Center or the Pentagon on September 11, 2001, to a control group of 55 search and rescue dogs that were not involved in the 9/11 response. Compared to controls, the deployed search dogs demonstrated mild changes in blood work and a higher incidence of radiographic cardiac abnormalities. Species differences may explain the lack of pulmonary findings in the dogs. These dogs may provide early evidence of nonpulmonary complications of the 9/11 response. Continued surveillance of all responders is warranted.**

Introduction

The September 11, 2001, terrorist attacks on the World Trade Center (WTC) resulted in over one million tons of debris covering 16 acres and massive clouds of particulates and toxins. Numerous environmental hazards including particulate matter, asbestos, polycyclic aromatic hydrocarbons, metal compounds, dioxins, and volatile organic compounds were identified at the WTC site (Banauch, Dhala, & Prezant, 2005). Acute and delayed respiratory symptoms have afflicted the WTC emergency responders (Moline, Herbert, & Nguyen, 2006; Moscato & Yacoub, 2007; Reissman & Howard, 2008).

The Pentagon attack site was smaller and lacked the hazards associated with the massive crushing and combustion of the WTC but still posed a potential risk for responders. A U.S. Environmental Protection Agency (U.S. EPA) air monitoring summary from October 9, 2001, reported only trace levels of asbestos, volatile organic compounds, and other chemicals. U.S. EPA did, however, identify high concentrations of arsenic and antimony in the soot and ash (Lyman, 2003).

In addition to the estimated 40,000 emergency response personnel at the WTC site and over 8,000 responders at the Pentagon, an estimated 250–300 canines responded, including more than 55 dogs at the Pentagon (Otto, Downend, Serpell, Ziemer, & Saunders, 2004; Slensky, Drobatz, Downend, & Otto, 2004). These dogs served three main purposes: detection (e.g., live victims by search and rescue [S&R] dogs, human remains by cadaver dogs, and explosive devices by bomb dogs), patrol (e.g., site security by police dogs), and mental health support (e.g., therapy dogs). At the Fresh Kills Landfill on Staten Island, dogs were used to assist in locating human remains during the sifting and sorting of WTC debris.

Search dog teams started arriving at the WTC on September 11. None of the dogs from outside of the New York City Police Department or the New York-New Jersey Port Authority were present during the tower collapse. The plume of dust, smoke, and toxic components generated by the collapse of the WTC was a major risk factor for human pulmonary complications. The second-highest risk period for both acute and chronic respiratory symptoms in humans occurred during the first two days following the collapse (Herbert et al., 2006; Prezant et al., 2002). Approximately half of the dogs responding to the WTC arrived within the first two days (Otto et al., 2004). The rain on September 14, 2001, likely helped to at least temporarily reduce the overall airborne level of pollutants in lower Manhattan; however, the rain also may have altered the composition of the dusts to which the responders were exposed. In the immediate work zone (Ground Zero), the unrelenting digging and moving of rubble and the uncontained fires resulted in persistent exposure to airborne toxins and particulates.

Although working dogs arrived at the Fresh Kills Landfill on and after September

TABLE **1**

1a – Group Descriptive Data

Characteristic	Deployed (*n* = 95)	Control (*n* = 55)	*p*-Value
Age (yrs.)	5.0 (3.0–7.0)	4.0 (2.0–6.0)	*p* = .019
Sex	54 males 41 females	31 males 24 females	*p* = .909
Breeds represented	11 pure and 8 mixed	14 pure and 1 mixed	N/A
Weight (kg)	31.2 ± 7.2	32.6 ± 7.7	*p* = .294
Geographic distribution	22 states	16 states and Canada	N/A

1b – Detailed Breed Comparisons

Breed	Deployed (*n* = 95)	Control (*n* = 55)
Airedale Terrier		2
Australian Cattle Dog		2
Australian Shepherd	4	
Beauceron	1	
Belgian Malinois		2
Belgian Tervuren	1	1
Bloodhound	7	1
Border Collie		1
Doberman Pinscher	1	
English Springer Spaniel	1	
German Shepherd	30	25
German Short-haired Pointer		1
Giant Schnauzer	1	
Golden Retriever	12	2
Hovawart		2
Keeshond		
Labrador	28	12
Louisiana Catahoula Leopard Hound		1
Mixed breed	8	1
Newfoundland		1
Rottweiler	1	1

17, the constant sifting and sorting of debris from both the WTC and pre-existing waste continuously aerosolized particulate matter and toxins. At the Pentagon, where about half of the dogs arrived on September 11, personal protective gear and respiratory protection requirements for the human responders were enforced. The use of respiratory protection at the WTC, particularly in the early days of the response, was variable. Regardless of the site, the S&R dogs were not equipped with respiratory protection and foot protection was only used for a limited number of dogs working the site perimeter.

The manifestation of pulmonary disease in workers responding to the WTC disaster has prompted great concern and speculation about long-term hazards from environmental exposure. The risks and long-term effects of response to the Pentagon (Lyman, 2003) have not had such an obvious manifestation. The S&R dogs shared the exposure risks with the human workers.

Companion animals, particularly dogs, may serve as sentinels of human disease due to several similarities between humans and dogs (van der Schalie et al., 1999), including genetics (Lindblad-Toh et al., 2005), physiology, shared diseases, and a common environment. As dogs age, they can develop cancer, heart failure, and dementia—diseases that take decades to manifest in humans. Given the condensed lifespan of the dog, shared environmental hazards may result in clinical manifestations in dogs long before they appear in humans.

Evaluation of the health of the dogs that responded to and were exposed to the hazards associated with these disasters provides information that may help minimize morbidity in future disasters. Importantly, morbidity or mortality in these dogs may provide valuable information for human health care and prevention. The results of annual evaluations that were initiated in October 2001 and continued through September 2006 as part of a medical surveillance program to monitor these dogs are reported here.

Methods

Dogs and Handlers

Handlers of S&R dogs that were deployed to the WTC, Fresh Kills Landfill, and Pentagon disaster sites were identified and contacted for enrollment in a health and behavioral study of their dogs as previously described (Otto et al., 2004; Slensky et al., 2004). The deployed cohort consisted of the S&R dogs that worked at one of the three disaster sites. Dogs that did not deploy to the 9/11 sites, but had similar S&R background and training, constituted the control cohort. Characteristics of the dogs are provided in Tables 1a and 1b.

Data Collection

Handlers completed consent forms to participate in the study and the forms were in compliance with the Institutional Animal Care and Use Committee and Institutional Review Board (IRB) committees at the University of Pennsylvania. The recruitment period began in October 2001 and ended in June 2002. After the recruitment and initial data collection period of Year 1 that ended on September 10, 2002, subsequent data (surveys and samples) collection periods ran from September 11, 2002, until September 10, 2003 (Year 2); September 11, 2003, until September 10, 2004 (Year 3); September 11, 2004, until September 10, 2005 (Year 4); and September 11, 2005, until September 10, 2006 (Year 5).

Surveys

Information from handlers was collected via survey instruments as previously described (Otto et al., 2004; Slensky et al., 2004). Subsequent health surveys for Years 2 through 5 requested complete contact information for the handler and the dog's veterinarian as well as medical history for the previous year (or since the last survey was completed). The health surveys included questions about the dog's current status in search and rescue (i.e., active vs. retired), as well as training and deployment activity during the previous year or since the last survey was completed.

Blood Samples

Due to the international distribution of dogs participating in the study, deployed and control dogs were evaluated annually by their local veterinarian. Blood and serum samples were obtained from the dogs and shipped overnight to the University of Pennsylvania for analysis. Complete blood counts and serum biochemical analyses were performed by the Clinical Pathology Laboratory at the Matthew J. Ryan Veterinary Hospital at the University of Pennsylvania.

Chest Radiographs

Pulmonary function testing is impractical in conscious dogs and not widely available. Therefore assessment of the respiratory system was limited to owner report and complete thoracic radiographs (right and left lateral and ventrodorsal or dorsoventral). Radiographs were obtained annually by the dog's local veterinarian and shipped to the University of Pennsylvania for evaluation by veterinary radiologists who were blinded to the study groups. Serial analysis of the first five years of radiographs was completed by a board-certified radiologist who was also blinded to the study groups. Each radiograph was scored for abnormalities in four categories: pulmonary, cardiac, musculoskeletal, and other.

Mortality

The cause of death was recorded for any dog that died or was euthanized during the study period. A full necropsy was requested. The gross examination was performed by the attending veterinarian and designated samples were shipped to Michigan State University for histopathologic analysis. If the handler failed to have a necropsy performed, the cause of death was recorded according to the attending veterinarian's diagnosis or as reported by the handler. The detailed results of the postmortem analysis in dogs that died or were euthanized during the initial five-year period have been reported (Fitzgerald, Rumbeiha, Emmett Braselton, Downend, & Otto, 2008).

Statistical Analyses

Health Survey

Fisher's exact test was used to test for an association between a medical condition and whether or not a dog was deployed after 9/11. Fisher's exact test was also used to test for an association between a medical problem or surgical procedure and whether or not the dog was deployed. Medical or surgical conditions were then sorted into 17 categories for each dog during the entire study period, and Fisher's exact test was again used to test for an association. Logistic regression was used to determine if the effect of deployment was significantly related to having a urology problem after controlling for the canine's age.

Hematology and Chemistry

Mixed linear models were used to evaluate mean differences for each of the hematologic and serologic outcomes and included a random subject effect and fixed effects of age, survey time period, deployed (yes/no), and the interaction of time period and deployment. Data was transformed to a normal distribution if necessary before being included in the model.

Radiograph Scores

For each of the three systemic categories (pulmonary, cardiac, musculoskeletal), the proportions of dogs exhibiting radiographic abnormalities were compared between the deployed and the control groups using the Chi-square test of independence. Fisher's exact test was used when any expected frequency was less than two, or if more than 20% of the expected frequencies were less than five.

Mortality

The proportion of dogs in each of the groups that died during the study period was compared using the Chi-square test of independence. Kaplan-Meier survival analyses were performed to test for homogeneity of survival curves over time for deployment (yes/no) and for the different deployment locations. The differences in length of survival from the deployment dates were determined by using the log rank tests of significance. Proportional hazards regression was performed to determine if an association existed between deployment, age, and survival. Dogs that did not die during the study period, or that were lost to follow up, were considered censored for the proportional hazards regression analysis.

Results

Search Dog Characteristics

Of the 216 deployed individuals that were identified and contacted, complete data was collected prior to the close of the first year of the study on 95 dogs (partial data from two additional dogs were included in the previous report after one year [Otto et al., 2004]). Fifty-five of the 114 control dog handlers that were contacted completed the required data to be included.

The overall survey completion rate decreased over time for both groups. For the first study period, 81.9% of deployed handlers completed surveys. For periods 2–4, the response rates decreased to 64.8%, 67.1%, and 61%, respectively. For the four study periods, control handler survey completion rates were 76.4%, 75.9%, 64.6%, and 70.5%, respectively. All handlers that enrolled during study period 0 were given the opportunity to participate every subsequent year that their dog was alive. Some handlers did not provide complete data for each year (e.g., completed surveys Years 0, 2, and 4 only) and therefore participation rates fluctuated. The overall attrition rate was not different between groups ($p = .492$).

Of the dogs for which data were provided, the average annual retirement rate was 14% for deployed dogs and 12% for controls. The highest single retirement time was in the first year and the most common cause for retirement was age of the dog. Overall, the median age of retirement was seven years for control dogs and nine years for deployed dogs; this difference did not reach significance. Dogs retired due to age were 10 years old on average.

TABLE **2**

Summary of Medical and Surgical Conditions Over All Time Periods

Condition	Deployed Dogs (*n* = 95)	Control dogs (*n* = 55)	*p*-Value*
Dermatologic	17 (17.9%)	9 (16.4%)	>.999
Gastrointestinal	26 (27.4%)	11 (20.0%)	.334
Infectious (vectorborne)	16 (16.8%)	4 (7.3%)	.135
Musculoskeletal	26 (27.4%)	17 (30.9%)	.709
Oncologic	18 (18.9%)	7 (12.7%)	.371
Respiratory	6 (6.3%)	1 (1.8%)	.423
Toxicologic	5 (5.3%)	4 (7.3%)	.725
Traumatic	27 (28.4%)	22 (40.0%)	.153
Urologic	18 (18.9%)	3 (5.5%)	.027

* Fisher's exact test.

Time of Arrival and Location of Deployed Dogs

Since air transportation was limited on September 11, 2001, and the days immediately after, search dog teams from the west (California, Texas) and far south (Florida), although deployed earlier, did not arrive at the WTC until after September 14. Thus the actual number of dogs that were exposed to the environmental hazards is smaller than previously reported. The highest risk period for pulmonary complications was during the time of the building collapse (Prezant et al., 2002); no dog in this study was present at that time. The second-highest risk period at the WTC was considered to be the subsequent two days, or until it rained on September 14. Of the 60 study dogs that searched at the WTC, 28 arrived prior to September 14. The time of highest risk at the Pentagon is not known. The first rain also occurred in Washington, DC, on September 14. Of the 23 study dogs that searched at the Pentagon, nine arrived before September 14. The Fresh Kills Landfill did not start receiving Ground Zero debris until September 12, and all 12 dogs in our study arrived at Fresh Kills after September 14.

The median length of time spent searching was 10 days (Otto et al., 2004), with a reported median of 24 hours of active searching. No difference occurred in median search time across the sites. Although active search time and therefore the most intensive exposure was limited to 24 hours, most WTC S&R teams were at Ground Zero for approximately 12-hour shifts. It can be estimated that these dogs were in a highly contaminated zone for approximately 120 hours.

Self-Reported Medical or Surgical Conditions

Owners were queried annually regarding the occurrence of several medical conditions. Since the surveys were collected at various times throughout the year from dog to dog, each medical condition was summarized at the end of the entire study period as to whether the dog ever had the medical condition since 9/11. This summary indicator was used to compare the deployed dogs to the control dogs for each medical condition. The summary of the medical conditions is for all 150 dogs whose handlers completed the initial survey. Although dogs withdrew from the study over time, no statistically significant differences existed in attrition rates between deployed and control dogs for any of the time periods. As a result of the combined effects of attrition and death, approximately 50% of the original deployed dogs remained at the end of the five-year study period and 56% of the original control dogs remained. All 150 dogs were included in the analysis.

The occurrence of any medical condition or surgical procedure since 9/11 of the previous year or since the last completed survey was determined. The most common categories were then summarized for each dog as to whether they had any medical or surgical condition that fell into one of these categories during the entire study period (Table 2). No significant differences existed between deployed and control for any of the categories listed with the exception of urological problems. More deployed dogs (18.9% [18/95]) had a medical or surgical urological condition during the study period compared to control dogs (5.5% [3/55]) (p = .027). Prior to the deployment period, deployed dogs were significantly more likely to have had lower urinary tract signs specifically (12/95 vs. 1/55, p = .023) or any urologic problem (16/95 vs. 1/55, p = .005) than were control dogs. Excluding the prior-to-deployment period, the incidence of urologic conditions (i.e., lower urinary tract signs, urinary incontinence, or prostatic problems) was not significantly different between deployed or control dogs during the deployment period or in the four subsequent evaluation periods.

Blood Chemistry and Hematology

Statistically higher concentrations of glucose, alkaline phosphatase, and cholesterol were detected in dogs that had been deployed, whereas control dogs had statistically higher serum potassium.

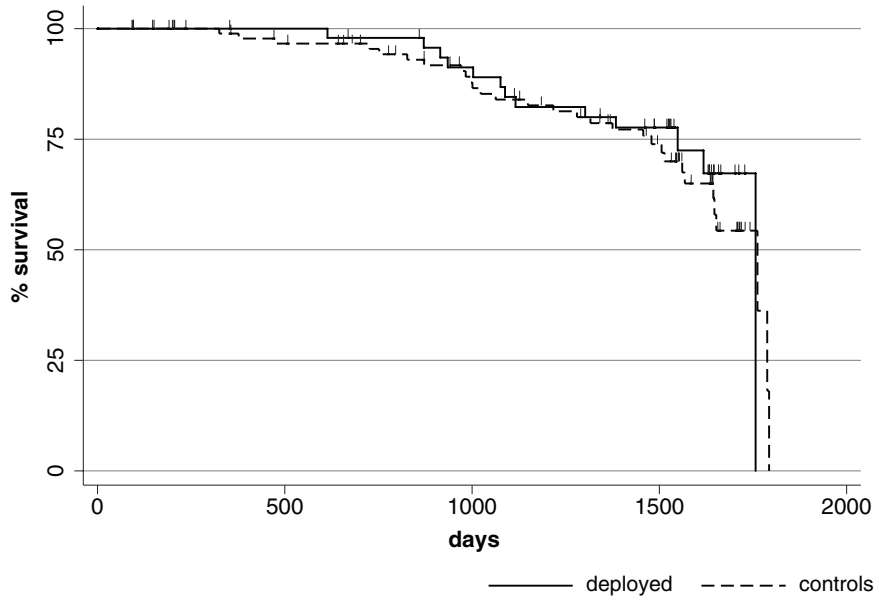
The glucose was outside the normal range (>112 mg/dL) in nine deployed dogs and four control dogs, but no dog had persistently elevated glucose and no dog had a glucose measurement higher than 129 mg/dL. Eleven deployed dogs had elevations in alkaline phosphatase (>174 U/L). Of the 10 deployed dogs with multiple measurements, seven had progressive increases in alkaline phosphatase over the study period. The highest value reported was 1,486 IU from a deployed dog undergoing glucocorticoid treatment for immune mediated hemolytic anemia. Subsequent values were within normal range for that dog. Only two control dogs had elevated alkaline phosphatase values; one dog had a transient elevation during period 2 and the other dog had elevated alkaline phosphatase in period 4. Twenty-two cholesterol measurements in 10 deployed dogs were elevated (>317 mg/dL). One control dog had a single elevated cholesterol measurement. Potassium was greater than the reference range (>4.9 mEq/dL) in 21 samples from 15 deployed dogs and in 28 samples from 21 control dogs. No dog in either group was hypokalemic at any time. The biochemical changes were independent of age.

No dog developed diabetes, and the mild elevations in blood glucose may have been a result of stress associated with blood collection or lack of fasting. The alkaline phosphatase elevations may have been associated with exogenous or endogenous (hyperadrenocorticism) steroids or concurrent hepatic disease. Primary hypercholesterolemia is uncommon in dogs and may have been associated with endocrine disease, especially hypothyroidism (Panciera, 1994). Recommendations were made to further evaluate these dogs; however, they were not tested for endocrine diseases as part of our study. The higher serum potassium values in the control dogs may have been related to artifact from hemolysis; no dog reported clinical signs or medical conditions associated with hyperkalemia.

Hematologic findings were not different between deployed and control dogs; however, based on subgroup analysis, white blood cell and neutrophil counts were significantly higher in dogs deployed to the Fresh Kills Landfill compared to the WTC ($p < .001$ and $p = .010$, respectively). In addition, neutrophil counts from dogs at the landfill were higher than control dogs ($p = .001$). The peripheral eosinophil counts, which can be increased in dogs with experimentally induced asthma (Collie, DeBoer, Muggenburg, & Bice, 1997), were actually significantly lower in deployed dogs (Otto et al., 2004).

FIGURE **1**

Kaplan Meier Survival Curve



Compares survival of search and rescue dogs responding to the 9/11/01 terrorist attacks to survival of search and rescue dogs that did not respond. Hash marks represent censored dogs.

Radiographs

No significant difference existed in the relative frequency of pulmonary or musculoskeletal lesions in the radiographs of deployed dogs compared to control dogs. Radiographic evidence of pulmonary abnormalities (predominately identified as diffuse bronchial and diffuse bronchointerstitial patterns) were present in 27.8% of deployed dogs versus 25.0% of control dogs ($p = .916$). Radiographs are an insensitive tool to detect changes in pulmonary function. Although pulmonary function testing was not available, retirement rate from search work was used as a surrogate for evidence of decreased respiratory function and was not different between groups. Allergic disease in dogs typically manifests as skin or ear conditions rather than as pulmonary conditions; however, the incidence of ear and skin conditions was not different between the control and deployed dogs.

Musculoskeletal abnormalities were diagnosed radiographically in 54.2% of deployed dogs and 46.7% on control dogs ($p = .572$). The most prevalent musculoskeletal injuries were arthritis in the appendicular skeleton and vertebral spondylosis. Of the 125 sets of radiographs that were evaluated, 28 control dogs and 40 deployed dogs had one or both of these musculoskeletal conditions.

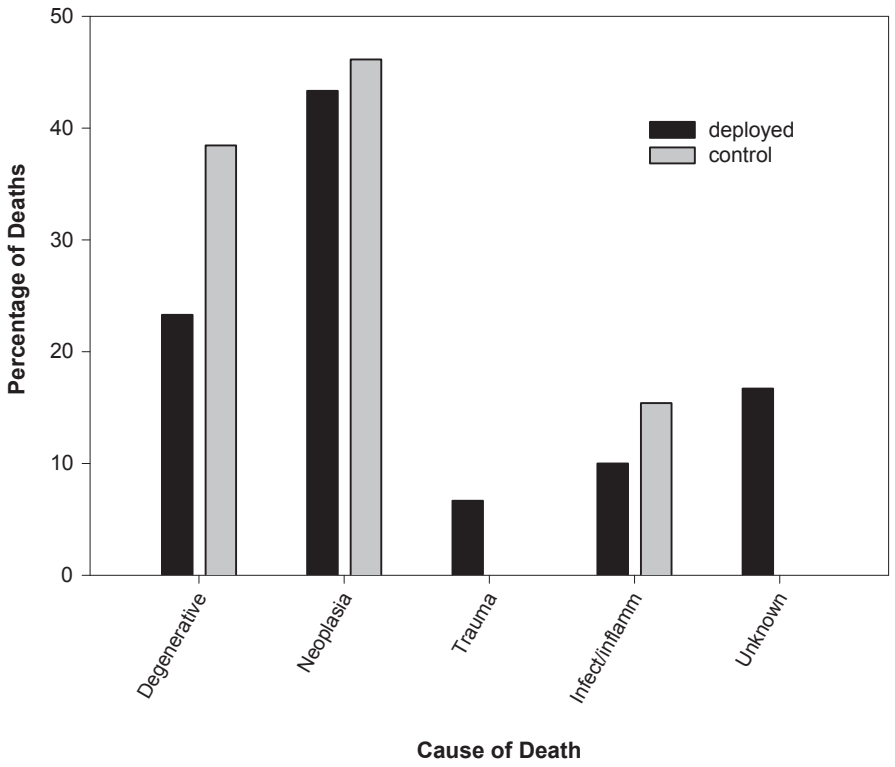
Radiographic cardiac abnormalities were significantly more likely to be diagnosed in deployed vs. control dogs (9.5% vs. 0.0%, respectively; Fischer's exact $p = .032$). Of the seven deployed dogs with cardiac lesions, five had mild left-sided cardiomegaly with atrial involvement, one had right-sided cardiomegaly, and one had mild generalized cardiomegaly. None of these dogs had evidence of congestive heart failure at the time of radiographs. Of the two dogs with radiographic abnormalities that subsequently died, one had histopathologic evidence of degenerative cardiomyopathy. An additional three dogs, which had incomplete radiologic evaluation, also had histopathologic evidence of cardiomyopathy. No control dogs had histopathologic evidence of cardiomyopathy. The incidence of cardiomyopathy was not significantly different between groups, but the number of cases evaluated is small.

Mortality

The proportion of deployed dogs that died during the follow-up period (31.6% [30/95]) was similar to the proportion of control dogs (23.6% [13/55]) ($p = .300$). The proportions of deaths at the WTC, Pentagon, and Fresh Kills Landfill were 30.4%, 31.7%, and 33.3%, respectively. The survival curves for deployed and control dogs were not significantly different ($p = .639$) (Figure 1), nor were they different for deployment locations ($p = .919$). In multivariate proportional hazards regression, age significantly increased the risk of death (hazard ratio = 1.34; $p < .001$), but deployment did not (hazard ratio = 0.85; $p = .634$). The cause of death was assigned to one of five categories; degenerative, neoplasia, infectious/inflammatory, trauma, and unknown (Figure 2, Table 3). The degenerative group was composed of dogs that the owners elected to euthanize due to progressive deterioration in attitude or perceived quality of life or organ degenerative disease (e.g., cardiomyopathy or degenerative myelopathy). Neoplasia was categorized by the body system affected (Table 3). Both dogs that died of trauma

FIGURE **2**

Categorical Cause of Death in Deployed and Control Dogs



Infect/inflamm = Infectious/inflammatory

sustained blunt trauma from a motor vehicle. Ischemic disease was categorized in the infectious/inflammatory category. The unknown category included all cases in which a necropsy was not performed and a premortem diagnosis was unable to categorize the etiology. For example, seizures (n = 1) could have been from degenerative disease, neoplasia, or infectious/inflammatory processes. For all dogs that underwent necropsy, detailed reports of the post mortem analyses has been published elsewhere (Fitzgerald et al., 2008).

Discussion

In these two cohorts of S&R dogs, response to the September 11, 2001, terrorist attacks has not resulted in detectable differences in most parameters studied. No long term follow-up of dogs responding to previous disasters has occurred. After the Oklahoma City bombing, the acute morbidity and short-term effects were reported (Duhaime, Norden, Corso, Mallonee, & Salman, 1998). Four other reports of follow-up in dogs responding to the 9/11 attacks are available. One study reported the acute morbidity associated with the response in 96 S&R dogs from across the country (Slensky et al., 2004). Similar to the acute morbidity reported for human rescue workers, the most common category of complaint was musculoskeletal (Berrios-Torres et al., 2003; Slensky et al., 2004), which included cuts, scrapes, abrasions, sprains, strains, and fractures. Surprisingly, despite the lack of protective gear, only four dogs sustained injuries sufficient to require sutures, and no dog sustained any fractures. In humans, respiratory complaints were the second most common problem, whereas the incidence of respiratory signs in this group of dogs was very low (incidence rate: 2.1 events/1,000 search hours) (Slensky et al., 2004) and was not different across the three sites (WTC, landfill, and Pentagon).

A recent report characterized the acute and long-term morbidity in a group of 27 New York City Police Department (NYPD) dogs that participated in the rescue and recovery response between September 11, 2001, and May 30, 2002 (Fox, Puschner, & Ebel, 2008). This group of dogs did have respiratory and ocular morbidity, which was likely associated with the fact that they arrived at the WTC earlier on September 11 than the group of dogs responding from around the country. Interestingly, the NYPD dogs did not show evidence of respiratory disease over the five years following the response (Fox et al., 2008). In 97 S&R dogs evaluated one year after the response, subtle blood work changes including increased immunoglobulin, bilirubin, and alkaline phosphatase were found in the deployed dogs compared to a cohort of S&R dogs that did not respond to the 9/11 attacks (Otto et al., 2004). Compared to the control dogs, no difference existed in the incidence of medical or surgical problems in the deployed dogs. Neither group had radiographic evidence of pulmonary disease (Otto et al., 2004). In a pathology study of S&R dogs (18 deployed and five control) that died between October 2002 and September 11, 2006, pulmonary anthracosis and particulate matter were commonly found in the lungs of both groups. No primary pulmonary neoplasia was identified in the deployed dogs (Fitzgerald et al., 2008). The most common type of neoplasia in the deployed dogs was hematopoietic (two dogs with lymphosarcoma, one with multiple myeloma, and one with tonsilar squamous cell carcinoma).

The significantly higher incidence of radiographic cardiac abnormalities in deployed dogs was unexpected. Cardiomegaly can result from a variety of causes and can be right-sided, left-sided or generalized. The one deployed dog with generalized cardiomegaly was a Labrador Retriever that eventually died (in 2007) of cardiomyopathy. Dilated cardiomyopathy is a recognized problem in Dobermans, Boxers, and giant breed dogs (Tidholm & Jonsson, 2005). One of the deployed dogs with right-sided cardiomegaly was a Doberman cross that developed cardiomyopathy, which was confirmed on postmortem examination. Three additional deployed dogs without radiographic changes (a Doberman, a German Shepherd, and a German Shepherd cross) were diagnosed on postmortem examination with degenerative cardiomyopathy. No control dogs had either radiographic or postmortem evidence of cardiac abnormali-

ties. Cardiomyopathy in dogs has been attributed to a variety of genetic and environmental factors and could be the manifestation of nutritional deficiencies, immunologic responses, infectious disease, or toxin exposure. Dogs chronically exposed to air pollution had evidence of histopathologic cardiac changes (Calderon-Garciduenas, Gambling et al., 2001) and inhaled particulate matter has been associated with increased cardiovascular morbidity in humans (Brook et al., 2004). Continued surveillance, with attention to cardiac morbidity, is warranted.

The absence of notable long-term morbidity in these dogs, particularly the lack of respiratory signs, is dramatically different than the findings in the human rescue workers. The earliest manifestation was the "WTC cough" (Prezant et al., 2002). Over time, the incidence of reactive airway disease and abnormal pulmonary function has been recognized in up to 70% of workers evaluated (Herbert et al., 2006). The risk associated with pulmonary signs was directly associated with the time of arrival at the WTC (Banauch et al., 2006; Prezant et al., 2002). The early use of respiratory protection was sporadic (Banauch et al., 2006). Regardless of arrival time, the dogs did not have any respiratory protection. During active scenting, dogs breathe through their noses, which may have allowed for better filtering of the large particulates compared to their mouth breathing handlers. Conversely, when dogs pant to maintain their body temperature, dead space (i.e., upper airway) ventilation increases (Robertshaw, 2006), which may have increased exposure of the tracheobronchial region to particulates.

Although not measured, the toxins and particulate matter in the air were thought to be at their highest level during the collapse of the towers. The air quality remained poor with high particulate matter until the rains of September 14 (Lioy & Georgopoulos, 2006). Almost half of the deployed dogs working in New York City arrived at the WTC before September 14, during the period of moderate risk. The Pentagon response has not been associated with physical morbidity.

Several reports cite an increased incidence of cardiovascular morbidity following the 9/11 terrorist attacks (Allegra, Mostashari, Rothman, Milano, & Cochrane, 2005; Feng, Lenihan, Johnson, Karri, & Reddy, 2006; Holman et al., 2008; Ornato, Muller, Froelicher, & Kloner, 2007). These reports attributed the increased incidence of myocardial infarction and other cardiac complaints to increased psychological stress (Allegra et al., 2005; Feng et al., 2006; Holman et al., 2008; Ornato et al., 2007). In dogs, myocardial infarction is rare (Driehuys, Van Winkle, Sammarco, & Drobatz, 1998). Although posttraumatic stress disorder is not recognized in dogs, anxiety can lead to other abnormal canine behaviors (Herron et al., 2008). We have no evidence of an increased incidence of anxiety disorders in the deployed dogs (Otto et al., 2004). The cardiac changes are therefore unlikely related to stress and may portend a previously unrecognized cardiac risk associated with the response to 9/11.

The difference between the respiratory morbidity in the canine and human rescue workers was unexpected, particularly given the high incidence of respiratory signs in the people and the lack of respiratory protection in the dogs. Canine and human pulmonary anatomies are similar, and experimental studies of inhaled particulate matter show similar deposition and clearance patterns in each species (Schlesinger, 1989; Snipes, 1989). In addition, dogs have been used as sentinels of both pulmonary and cardiac effects of chronic exposure to air pollution (Calderon-Garciduenas, Gambling et al., 2001; Calderon-Garciduenas, Mora-Tiscareno et al., 2001). Despite the structural similarities, dogs have relatively larger conduct-

TABLE **3**

Primary Cause of Death in Study Dogs

Characteristic/Cause	Deployed (*n* = 30)	Control (*n* = 13)
Site	WTC = 19 Landfill = 4 Pentagon = 7	N/A
Age at death (years) (*p* = .251)	9.9 ± 3.0	11.1 ± 2.8
Cause of death (*n* = confirmed by necropsy)	*n* = 15	*n* = 5
Degenerative	8 (*n* = 6)	5 (*n* = 3)
Neurologic	5	2
Musculoskeletal	0	3
Cardiac	3 (one additional dog with a primary diagnosis of neoplasia)	0
Neoplasia	13 (*n* = 6)	6 (*n* = 2)
Hematopoietic	4	0
Vascular	2	1
Urogenital	2	1
Musculoskeletal	2	1
Hepatic/Gastrointestinal	1	1
Skin	1 (concurrent cardiomyopathy)	0
Neurologic	1	
Pulmonary	0	1
		1
Trauma	2 (*n* = 1)	0
Infectious/inflammatory	2 (*n* = 2)	2 (*n* = 0)
Gastrointestinal	1	2
Musculoskeletal	1	0
Unknown	5 (*n* = 5)	0
Neurologic	3	
Gastrointestinal	1	
Urogenital	1	

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ing airways and efficient collateral airways that may minimize concentrated exposure to irritants and allergens (Bice, Seagrave, & Green, 2000; Kirschvink & Reinhold, 2008). Dogs are also highly resistant to the development of asthma or reactive airway disease. In experimental settings, dogs have been used as a model of asthma; however, the model requires complex exposure regimens or uniquely bred strains (Hirshman, Malley, & Downes, 1980; Kepron, James, Kirk, Sehon, & Tse, 1977). In these experimental models, airway hyper-reactivity can be documented (Barrett, Rudolph, Bowen, & Bice, 2003), but clinical evidence of pulmonary compromise has not been demonstrated (E.G. Barrett, personal communication, October 31, 2008). In Alaskan sled dogs, cold-induced pulmonary inflammation has been documented in dogs that have completed an 1,100 mile race, suggesting that despite cytologic evidence of inflammation and mucous accumulation, performance was minimally compromised (Davis et al., 2002).

In addition to the resistance to clinical signs of asthma or reactive airway disease, the longer nasal passages and necessity for nasal breathing during scent work may have more effectively filtered the particulate matter and toxins. In the absence of respiratory protection, mouth-breathing human responders were likely to have an increased respiratory accumulation of the larger (>2.5 µm) particles, particularly in the tracheobronchial region (Schlesinger, 1989). The particulate matter at the WTC was predominantly composed of large particles (>10 µm) (Chen & Thurston, 2002). In experimental studies, mouth-breathing humans are more likely than dogs to deposit these large particles in the respiratory tract (particularly in the tracheobronchial tree) (Schlesinger, 1989), providing one potential reason for the difference in pulmonary signs between the species. Dogs also lack many of the complicating behaviors (e.g., smoking). Compared to firefighters, S&R dogs have a much lower occupational hazard, a factor that may exacerbate or contribute to symptoms in people.

Although these S&R dogs do not appear to be sentinels of airway hyper-reactivity, the fact that they shared the same environmental exposure as the human rescue workers suggests that other effects of the response may be evident in the dogs before they are seen in people. The dogs in our study were only exposed for an average of 10 days. While this is a relatively low exposure duration, the majority of dogs were part of urban S&R teams, and the human team members had identical response and exposure times as the dogs. The relatively small number of dogs on this study—95 deployed dogs, with only 73 at the WTC or landfill—would make it impossible to show an association with rare or infrequent conditions that were a result of the exposure.

Although handler reporting is likely to introduce bias, the emphasis on rescue worker pulmonary effects may have made the deployed dog handlers more vigilant in monitoring for respiratory signs. The ideal would have been a more standardized physical examination including pulmonary function testing, but that was not practical.

In conclusion, the dogs that responded to the 9/11 terrorist attacks did not show respiratory symptoms either acutely during deployment or over the subsequent five years. This difference between the human and canine workers is likely a result of difference in both anatomy and physiology. Overall, deployed dogs had minor changes in blood work. Whether these signs were associated with stress, exertion, infection, or toxin clearance cannot be determined. Radiographic evaluation of the heart identified more abnormalities in the deployed dogs versus the controls and four deployed dogs had confirmed cardiomyopathy. The death rate in both groups was similar, as was the diagnosis of cancer. Continued surveillance of all responders is warranted, realizing that the S&R dogs may be sentinels of other or unexpected sequelae to the 9/11 response.

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References

Allegra, J.R., Mostashari, F., Rothman, J., Milano, P., & Cochrane, D.G. (2005). Cardiac events in New Jersey after the September 11, 2001, terrorist attack. *Journal of Urban Health, 82*(3), 358–363.

Banauch, G.I., Dhala, A., & Prezant, D.J. (2005). Pulmonary disease in rescue workers at the World Trade Center site. *Current Opinion in Pulmonary Medicine, 11*(2), 160–168.

Banauch, G.I., Hall, C., Weiden, M., Cohen, H.W., Aldrich, T.K., Christodoulou, V., Arcentales, N., Kelly, K.J., & Prezant, D.J. (2006). Pulmonary function after exposure to the World Trade Center collapse in the New York City Fire Department. *American Journal of Respiratory & Critical Care Medicine, 174*(3), 312–319.

Barrett, E.G., Rudolph, K., Bowen, L.E., & Bice, D.E. (2003). Parental allergic status influences the risk of developing allergic sensitization and an asthmatic-like phenotype in canine offspring. *Immunology, 110*(4), 493–500.

Berrios-Torres, S.I., Greenko, J.A., Phillips, M., Miller, J.R., Treadwell, T., & Ikeda, R.M. (2003). World Trade Center rescue worker injury and illness surveillance, New York, 2001. *American Journal of Preventive Medicine, 25*(2), 79–87.

Bice, D.E., Seagrave, J., & Green, F.H.Y. (2000). Animal models of asthma: Potential usefulness for studying health effects of inhaled particles. *Inhalation Toxicology, 12*(9), 829–862.

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References

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Brook, R.D., Franklin, B., Cascio, W., Hong, Y., Howard, G., Lipsett, M., Luepker, R., Mittleman, M., Samet, J., Smith, S.C., Jr., & Tager, I. (2004). Air pollution and cardiovascular disease: A statement for health care professionals from the expert panel on population and prevention science of the American Heart Association. *Circulation, 109*(21), 2655–2671.

Calderon-Garciduenas, L., Gambling, T.M., Acuna, H., Garcia, R., Osnaya, N., Monroy, S., Villarreal-Calderon, A., Carson, J., Koren, H.S., & Devlin, R.B. (2001). Canines as sentinel species for assessing chronic exposures to air pollutants: Part 2—Cardiac pathology. *Toxicological Sciences, 61*(2), 356–367.

Calderon-Garciduenas, L., Mora-Tiscareno, A., Fordham, L.A., Chung, C.J., Garcia, R., Osnaya, N., Hernandez, J., Acuna, H., Gambling, T.M., Villarreal-Calderon, A., Carson, J., Koren, H.S., & Devlin, R.B. (2001). Canines as sentinel species for assessing chronic exposures to air pollutants: Part 1—Respiratory pathology. *Toxicological Sciences, 61*(2), 342–355.

Chen, L.C., & Thurston, G. (2002). World Trade Center cough. *The Lancet, 360*(Supplement 1), S37–S38.

Collie, D.D., DeBoer, D.J., Muggenburg, B.A., & Bice, D.E. (1997). Evaluation of association of blood and bronchoalveolar eosinophil numbers and serum total immunoglobulin E concentration with the expression of nonspecific airway reactivity in dogs. *American Journal of Veterinary Research, 58*(1), 34–39.

Davis, M.S., McKiernan, B., McCullough, S., Nelson, S., Jr., Mandsager, R.E., Willard, M., & Dorsey, K. (2002). Racing Alaskan sled dogs as a model of "ski asthma." *American Journal of Respiratory and Critical Care Medicine, 166*(6), 878–882.

Driehuys, S., Van Winkle, T.J., Sammarco, C.D., & Drobatz, K.J. (1998). Myocardial infarction in dogs and cats: 37 cases (1985–1994). *Journal of the American Veterinary Medical Association, 213*(10), 1444–1448.

Duhaime, R.A., Norden, D., Corso, B., Mallonee, S., & Salman, M.D. (1998). Injuries and illnesses in working dogs used during the disaster response after the bombing in Oklahoma City. *Journal of the American Veterinary Medical Association, 212*(8), 1202–1207.

Feng, J., Lenihan, D.J., Johnson, M.M., Karri, V., & Reddy, C.V.R. (2006). Cardiac sequelae in Brooklyn after the September 11 terrorist attacks. *Clinical Cardiology, 29*(1), 13–17.

Fitzgerald, S.D., Rumbeiha, W.K., Emmett Braselton, W., Downend, A.B., & Otto, C.M. (2008). Pathology and toxicology findings for search-and-rescue dogs deployed to the September 11, 2001, terrorist attack sites: Initial five-year surveillance. *Journal of Veterinary Diagnostic Investigation, 20*(4), 477–484.

Fox, P.R., Puschner, B., & Ebel, J.G. (2008). Assessment of acute injuries, exposure to environmental toxins, and five-year health surveillance of New York Police Department working dogs following the September 11, 2001, World Trade Center terrorist attack. *Journal of the American Veterinary Medical Association, 233*(1), 48–59.

Herbert, R., Moline, J., Skloot, G., Metzger, K., Baron, S., Luft, B., Markowitz, S., Udasin, I., Harrison, D., Stein, D., Todd, A., Enright, P., Stellman, J.M., Landrigan, P.J., & Levin, S.M. (2006). The World Trade Center disaster and the health of workers: Five-year assessment of a unique medical screening program. *Environmental Health Perspectives, 114*(12), 1853–1858.

Herron, M.E., Shofer, F.S., Reisner, I.R., Herron, M.E., Shofer, F.S., & Reisner, I.R. (2008). Retrospective evaluation of the effects of diazepam in dogs with anxiety-related behavior problems. *Journal of the American Veterinary Medical Association, 233*(9), 1420–1424.

Hirshman, C.A., Malley, A., & Downes, H. (1980). Basenji-Greyhound dog model of asthma: Reactivity to ascaris suum, citric acid, and methacholine. *Journal of Applied Physiology, 49*(6), 953–957.

Holman, E.A., Silver, R.C., Poulin, M., Andersen, J., Gil-Rivas, V., & McIntosh, D.N. (2008). Terrorism, acute stress, and cardiovascular health: A 3-year national study following the September 11th attacks. *Archives of General Psychiatry, 65*(1), 73–80.

Kepron, W., James, J.M., Kirk, B., Sehon, A.H., & Tse, K.S. (1977). Canine model for reaginic hypersensitivity and allergic bronchoconstriction. *Journal of Allergy and Clinical Immunology, 59*(1), 64–69.

Kirschvink, N., & Reinhold, P. (2008). Use of alternative animals as asthma models. *Current Drug Targets, 9*(6), 470–484.

Lindblad-Toh, K., Wade, C.M., Mikkelsen, T.S., Karlsson, E.K., Jaffe, D.B., Kamal, M., & et al. (2005). Genome sequence, comparative analysis and haplotype structure of the domestic dog. *Nature, 438*(7069), 803–819.

Lioy, P.J., & Georgopoulos, P. (2006). The anatomy of the exposures that occurred around the World Trade Center site: 9/11 and beyond. *Annals of the New York Academy of Sciences, 1076*, 54–79.

Lyman, F. (2003). *Messages in the dust. What are the lessons of the environmental health response to the terrorist attacks of September 11?* Retrieved June 15, 2010, from http://www.neha.org/pdf/messages_in_the_dust.pdf

Moline, J., Herbert, R., & Nguyen, N. (2006). Health consequences of the September 11 World Trade Center attacks: A review. *Cancer Investigation, 24*(3), 294–301.

Moscato, G., & Yacoub, M.R. (2007). World Trade Center disaster: Short- and medium-term health outcome. *Monaldi Archives for Chest Disease, 67*(3), 154–158.

Ornato, J.P., Muller, J.E., Froelicher, E.S., & Kloner, R.A. (2007). Task force II: Indirect and secondary cardiovascular effects of biological terrorism agents and diseases. *Circulation, 115*(12), 1672–1680.

Otto, C.M., Downend, A.B., Serpell, J.A., Ziemer, L.S., & Saunders, H.M. (2004). Medical and behavioral surveillance of dogs deployed to the World Trade Center and the Pentagon from October 2001 to June 2002. *Journal of the American Veterinary Medical Association, 225*(6), 861–867.

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References

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Panciera, D.L. (1994). Hypothyroidism in dogs: 66 cases (1987–1992). *Journal of the American Veterinary Medical Association, 204*(5), 761–767.

Prezant, D.J., Weiden, M., Banauch, G.I., McGuinness, G., Rom, W.N., Aldrich, T.K., & Kelly, K.J. (2002). Cough and bronchial responsiveness in firefighters at the World Trade Center site. *New England Journal of Medicine, 347*(11), 806–815.

Reissman, D.B., & Howard, J. (2008). Responder safety and health: Preparing for future disasters. *Mount Sinai Journal of Medicine, 75*(2), 135–141.

Robertshaw, D. (2006). Mechanisms for the control of respiratory evaporative heat loss in panting animals. *Journal of Applied Physiology, 101*(2), 664–668.

Schlesinger, R.B. (1989). Deposition and clearance of inhaled particles. In R.O. McClellan & R.F. Henderson (Eds.), *Concepts in inhalation toxicology* (pp. 163–192). New York: Hemisphere Publishing Corporation.

Slensky, K., Drobatz, K., Downend, A., & Otto, C. (2004). Deployment morbidity among search and rescue dogs from 9/11. *Journal of the American Veterinary Medical Association, 225*(6), 868–873.

Snipes, M.B. (1989). Species comparisons for pulmonary retention of inhaled particles. In R.O. McClellan & R.F. Henderson (Eds.), *Concepts in inhalation toxicology* (pp. 193–227). New York: Hemisphere Publishing Corporation.

Tidholm, A., & Jonsson, L. (2005). Histologic characterization of canine dilated cardiomyopathy. *Veterinary Pathology, 42*(1), 1–8.

van der Schalie, W.H., Gardner, H.S., Jr., Bantle, J.A., De Rosa, C.T., Finch, R.A., Reif, J.S., Reuter, R.H., Backer, L.C., Burger, J., Folmar, L.C., & Stokes, W.S. (1999). Animals as sentinels of human health hazards of environmental chemicals. *Environmental Health Perspectives, 107*(4), 309–315.

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Research paper

Generation and validation of canine single chain variable fragment phage display libraries

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ABSTRACT

Single chain variable region fragments (scFvs) are composed of an immunoglobulin (Ig) variable heavy (VH) and variable light (VL) chain joined by a flexible serine–glycine linker. They represent the smallest antibody fragments that maintain antigen specificity and they hold significant potential for therapeutic antigen targeting in vivo. Here we report on the design and validation of a series of degenerate primers that amplify the recombined variable regions of canine Ig heavy and light chain genes from lymphocyte cDNA. We show that these VH and VL amplicons can be randomly combined by a flexible linker using splicing by overlap extension PCR to form scFv constructs that can be expressed on the surface of M13 bacteriophage. To demonstrate that scFvs with specificity for previously encountered antigens are contained within these scFv phage display libraries we used simple panning procedures to isolate canine parvovirus (CPV) specific scFvs from a library made from the splenocytes of a dog immunized against CPV. These studies reveal the feasibility of this approach for generating diverse canine scFv libraries and pave the way toward future studies to isolate canine antigen-specific scFv of interest that may be tested as targeting agents for the treatment of infectious, inflammatory and neoplastic diseases in the dog.

1. Introduction

The ability to amplify the variable regions of immunoglobulin heavy and light chains from B lymphocytes and combine those chains to form single chain variable region fragments (scFv) allows the construction of libraries of Ab fragments that approximate the individual's immunological repertoire (Mao et al., 1999; Felding-Habermann et al., 2004). Combining this technique with phage display allows the generation of scFv phage display libraries that may be rapidly screened to identify and isolate Ag-specific targeting molecules for use in a wide range of biomedical applications (Lerner et al., 1991). These include therapeutic and diagnostic tumor

Abbreviations: CDR, complementarity determining region; CPV, canine parvovirus; CSCG, Canine Single Chain Gamma; CSCJK, Canine Single Chain Joining Kappa; CSCJLam, Canine Single Chain Joining Lambda; CSCK, Canine Single Chain Kappa; CSCLam, Canine Single Chain Lambda; CSCVH, Canine Single Chain Variable Heavy; FR, framework region; ORF, open reading frame; scFv, single chain variable fragment; VH, variable heavy chain; VL, variable light chain.

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Ag targeting, toxin and virus neutralization, blockade of cytokine–cytokine receptor interactions and inhibition of aberrant intracellular signaling pathways (Begent et al., 1996; Klimka et al., 1999; Haidaris et al., 2001; Posey et al., 2002; Felding-Habermann et al., 2004; Riano-Umbarila et al., 2005; Knackmuss et al., 2007; Pelat et al., 2007).

Degenerate primers designed to amplify the VH and VL regions of immunoglobulins and generate combinatorial scFv and Fab libraries have been described for many animals including humans, rats, mice, camels, rabbits, chickens and sharks (Ridder et al., 1995; Davies and Riechmann, 1996; Gao et al., 2002; Schluter et al., 2005; Foord et al., 2007; Sepulveda and Shoemaker, 2008). However, relatively little is known about the genetics of canine immunoglobulins, and the ability to generate combinatorial Ab libraries in the dog has not yet been described. Here we describe the design and validation of a set of degenerate primers for amplifying rearranged canine immunoglobulin VH (IgG) and VL chains. We show that these chains can be randomly combined to generate scFvs libraries that can be expressed on the surface of bacteriophage. Furthermore, we show that scFvs specific for the capsid antigens of canine parvovirus (CPV) can be selected and isolated from scFv phage display libraries generated from the splenocytes of a dog previously immunized against CPV using simple panning techniques.

The ability to generate scFv phage display libraries from the canine antigen-experienced Ig repertoire provides a useful tool for generating and isolating scFvs of canine origin that may have therapeutic relevance for the treatment of a number of different canine diseases. Furthermore, the scFv libraries that are based on the antigen-experienced Ig repertoire in this report may be used to provide useful information regarding the use of Ig genes during a natural immune response in the dog.

2. Materials and methods

2.1. Bioinformatics and sequence alignments

Predicted and/or cloned canine and human immunoglobulin sequences were used as queries in a discontiguous megablast search of the non-redundant nucleotide and genomic sequence databases and the non-redundant protein sequences in the high quality assembly of the canine genome (CanFam 7.6X). Nucleotide and amino acid sequence alignments were performed using the web-based multiple sequence alignment program CLUSTALW (http://www.ebi.ac.uk/clustalw/). Immunoglobulin V region sequences were analyzed using the NCBI Ig BLAST web-based program (http://www.ncbi.nlm.nih.gov/igblast/).

2.2. Total RNA isolation and cDNA synthesis

Animal studies were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee. Discarded splenic tissue was collected from four dogs previously vaccinated against CPV, that underwent therapeutic splenectomy for the treatment of ruptured splenic hemangiosarcoma at the University of Pennsylvania's School of Veterinary Medicine. Samples were immediately placed in RNAlater (Applied Biosystems, Foster City, CA) and stored at −80 °C. Total RNA was extracted from all samples using the RNeasy kit (Qiagen Inc., Valencia, CA). RNA quality was determined by gel electrophoresis and RNA quantity was determined by UV absorbance at 260 and 280 nm. Reverse transcription was performed using oligo dT and superscript II reverse transcriptase (Invitrogen Corp., Carlsbad, CA) according to the manufacturer's instructions.

2.3. Generation of canine VH and VL (λ and κ) chains using PCR

VH and VLλ and κ chains were amplified independently using the combination of primers described in Table 1. Each of the 9 VH forward primers was paired with the CSCG1234-B primer (9 separate reactions), each of the 16 VLλ forward primers was paired with each of the 2 VLλ reverse primers (32 separate reactions) and each of the 6 VLκ forward primers was paired with each of the 4 VLκ reverse primers (24 separate reactions). cDNA was amplified in PCR reactions that contained 60 pmol of sense and anti-sense primers, 10× PCR buffer, 150 mM of $MgCl_2$, 10 mM dNTPs and 0.5 U Taq DNA polymerase (Invitrogen Corp.). Cycling conditions were 94 °C for 5 min, followed by 30 cycles of 94 °C for 15 s; 56 °C for 15 s and 72 °C for 90 s, followed by a final extension at 72 °C for 10 min. Products were kept at 4 °C prior to analysis by gel electrophoresis.

2.4. Generation of canine single chain variable fragments

Products from the first round of PCR amplification were pooled into three groups (VH, VLλ and VLκ), and gel was purified using the QIAquick Gel Extraction kit (Qiagen Inc.). Purified products were analyzed on a 1% agarose gel and quantified using UV absorbance at 260 and 280 nm. Equimolar concentrations of VH and VLλ or VH and VLκ amplicons were randomly combined in a second round PCR reaction to generate separate VH–VLλ and VH–VLκ libraries using primers that anneal to conserved recognition sites incorporated into the 5′ ends of the VL forward and VH reverse primers (RSC-F and RSC-B, Table 1). VH and VL amplicons were able to be spliced together due to the presence of complementary overlapping sequences encoding an eighteen amino acid flexible glycine–serine linker (GGSSRSSSSGGGGSGGGG) incorporated into the 5′ region of all VH forward primers and the 3′ end of the VL reverse primers (Barbas, 2001). Briefly, each PCR reaction contained 100 ng of VH products and either 100 ng of VLλ products or 100 ng of VLκ products, 60 pmol of RSC-F and RSC-B primers, 10× Thermo buffer (New England Biolabs, Ipswich, MA), 10 mM dNTPs and 2 U Vent DNA polymerase (New England Biolabs). Cycling conditions were as follows: 94 °C for 5 min, followed by 30 cycles of 94 °C for 15 s; 56 °C for 30 s; 72 °C for 2 min, followed by a final extension of 72 °C for 10 min. Products were kept at 4 °C until analysis by gel electrophoresis. VH–VLλ and VH–VLκ scFvs were gel purified on a 2% agarose gel using the QIAquick Gel Extraction kit (Qiagen Inc.). Purified products were analyzed on a

Table 1
Nucleotide sequences of primers used to amplify canine immunoglobulin genes and construct scFv libraries (underlined regions indicate conserved linker sequence and second round primer binding sites).

Canine VH forward primers	
CSCVH1-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GTV CAR CTG GTG SAR TCT-3′
CSCVH2-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GTR MVD YTG GTG GAR TCT-3′
CSCVH3-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GRS GTG CAG CTG GTG GAG TCT-3′
CSCVH4-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GTR CAG CTG STG GAG WMT-3′
CSCVH5-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAR KWG CAR CTG GTG GAG YTT-3′
CSCVH6-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GGG CAG CTG GCG GAG TCT-3′
CSCVH7-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAR BTN MAR YTG GTN GAR WSN-3′
CSCVH8-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GTB CAR CTG GTR SAG TCT-3′
CSCVH9-F	5′-GGT GGT TCC TCT AGA TCT TCC TCC TCT GGT GGC GGT GGC TCG GGC GGT GGT GGG GAG GTG CAA CTG VWG RAG TCY-3′
Canine VH reverse primers	
CSCG1234-B	5′-CCT GGC CGG CCT GGC CAC TAG AAC CGA GGG GGC CGT GGT GGA-3′
Canine VL forward primers	
CSCLam1a-F	5′-GGG CCC AGG CGG CCG AGC TCG TGC TGA MTC MGC YRS SYT CD-3′
CSCLam1b-F	5′-GGG CCC AGG CGG CCG AGC TCR YSC TGA CTC ARM MGS CCT CM-3′
CSCLam1c-F	5′-GGG CCC AGG CGG CCG AGC TCG TSC TGA CTC AGC YDV CCT CA-3′
CSCLam1d-F	5′-GGG CCC AGG CGG CCG AGC TCG YGY TGA CYC ARC YRG CCT CM-3′
CSCLam2-F	5′-GGG CCC AGG CGG CCG AGC TCN BVY TGA CKC ARC CDD CCT CR-3′
CSCLam3-F	5′-GGG CCC AGG CGG CCG AGC TCG TGC TGW CWC AGC YGC CAT CM-3′
CSCLam4-F	5′-GGG CCC AGG CGG CCG AGC TCG TGC TGA CTC AGC CTC CYT C-3′
CSCLam5-F	5′-GGG CCC AGG CGG CCG AGC TCG RGY TGA CTC AGC YRC CWT C-3′
CSCLam6-F	5′-GGG CCC AGG CGG CCG AGC TCG GGY TGA ATC AGS CTY CCT C-3′
CSCLam7-F	5′-GGG CCC AGG CGG CCG AGC TCG TDN TVA CYC ARS MRV CMT CA-3′
CSCLam8-F	5′-GGG CCC AGG CGG CCG AGC TCT GCT GAC CCA GAC TCC AAG TG-3′
CSCLam9-F	5′-GGG CCC AGG CGG CCG AGC TCK CTG ACC CAG MCT MCA AGT GC-3′
CSCLam10-F	5′-GGG CCC AGG CGG CCG AGC TCG TRC TGA CYC ARC CKC CKT CW-3′
CSCLam11-F	5′-GGG CCC AGG CGG CCG AGC TCG TRM GSA AYC ARC CKC CKT CW-3′
CSCLam12-F	5′-GGG CCC AGG CGG CCG AGC TCC TGC TGA CYC ARC CKG CYT CW-3′
CSCLam13-F	5′-GGG CCC AGG CGG CCG AGC TCG TRC TGA AYC ARC CKC CKT CW-3′
Canine VL reverse primers	
CSCJLam1-B	5′-GGA AGA TCT AGA GGA ACC ACC GCC GAG GAC GGT CAG STG GGT SCC-3′
CSCJLam2-B	5′-GGA AGA TCT AGA GGA ACC ACC ACC WAG GAC GGT SAG YTS GRT TCC-3′
Canine VLκ forward primers	
CSCK1-F	5′-GGG CCC AGG CGG CCG AGC TCC AGA TGA CCC AGT CCC CAA-3′
CSCK24-F	5′-GGG CCC AGG CGG CCG AGC TCG TSA TGA YRC AGA CYC CAC-3′
CSCK34-F	5′-GGG CCC AGG CGG CCG AGC TCG TGA TGA CMC AGT CTC CAG-3′
CSCK4-F	5′-GGG CCC AGG CGG CCG AGC TCA YSM TGA CYC AGT KYC CAG-3′
CSCK5-F	5′-GGG CCC AGG CGG CCG AGC TCG TSA TGA YRC AGR CBC CAC-3′
CSCK6-F	5′-GGG CCC AGG CGG CCG AGC TCT GTC ATG ACA CAG ACC CCA-3′
Canine VLκ reverse primers	
CSCJK1-B	5′-GGA AGA TCT AGA GGA ACC ACC TTT GAG YTC CAC CTK GGT WCC-3′
CSCJK2-B	5′-GGA AGA TCT AGA GGA ACC ACC TTT GAG CTC CTC CTT GGT TCG-3′
CSCJK3-B	5′-GGA AGA TCT AGA GGA ACC ACC TTT GAG GTC CAC CTT GGT TCC-3′
CSCJK4-B	5′-GGA AGA TCT AGA GGA ACC ACC TTT KAT CTC CAV CTT GGT YCC-3′
Second round PCR reaction primers	
RSC-F	5′-GAG GAG GAG GAG GAG GAG GCG GGG CCC AGG CGG CCG AGC TC-3′
RSC-B	5′-GAG GAG GAG GAG GAG GAG CCT GGC CGG CCT GGC CAC TAG TG-3′
Sequencing primers	
5LC	5′-AAG ACA GCT ATG GCG ATT G-3′
DPSEQ	5′-AGA AGC GTA GTC CGG AAC GTC-3′

1% agarose gel and quantified using UV absorbance at 260 and 280 nm.

2.5. Cloning of PCR products into a phagemid vector

The phagemid vector, pComb3X (a kind gift of Dr. Carlos Barbas, Scripps Research Institute, La Jolla, CA) and generated scFvs were digested with the restriction endonuclease SfiI to generate complementary cohesive ends. Products were purified using the QIAquick Gel Extraction kit (Qiagen Inc.). The quality and quantity of each fragment were determined by gel electrophoresis and UV absorbance at 260 and 280 nm, respectively. Test ligation reactions were prepared using 140 ng of vector and either 70 or 140 ng of the generated scFvs to identify the optimal mass ratio of vector to scFv insert that would produce the most transformants. Test ligations were transformed into electrocompetent XL-1 Blue cells (Stratagene, LaJolla, CA) and incubated overnight on carbenicillin impregnated agar plates. Twenty-four hours later, the number of transformants per microgram of DNA vector was calculated from bacterial colony counts to determine transformation efficiency as previously described (Barbas, 2001). Transformation efficiencies of at least $1 \times 10^7/\mu g$ vector DNA

were required to proceed with large scale library ligations. Library ligation reactions were prepared using 1.4 μg pComb3X vector and 700 ng scFv insert (identified as the optimal mass ratio in test ligations).

2.6. DNA sequencing and analysis

Individual colonies were selected from VH–VLλ and VH–VLκ scFv libraries and grown overnight in carbenicillin selection media as described (Payne et al., 2005). DNA was extracted from these cultures using the Zyppy mini-prep DNA extraction system (Zymo Research Corp., Orange, CA). Plasmids were digested using SfiI and run on a 1% agarose gel to confirm the presence of a scFv insert. Plasmids containing the expected ~850 bp inserts were sequenced at the University of Pennsylvania's DNA sequencing core facility using the pComb3X-specific primers 5LC and DPseq (Table 1). Sequences were analyzed to identify open reading frames (ORF) and translated into protein sequences using the web-based, NCBI ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/). VH and VL sequences were used as input data in a BLAST search of the canine genome (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Using the human sequence subgroup program in the Kabat database, sequenced canine VH and VL (λ and κ) chains were assigned to family subgroups. The complementarity determining regions (CDR) and framework regions (FR) of each sequenced VH and VL chain were approximated using the human Ig BLAST program (http://www.ncbi.nlm.nih.gov/igblast/).

2.7. BstNI fragment analysis

Plasmid DNA was isolated from individual bacterial clones from titering plates of an unpanned scFv library. scFv inserts were amplified by PCR using pComb3X-specific primers and products were digested with BstNI as previously described (Marks et al., 1991). Digested products were analyzed on a 3% agarose gel.

2.8. scFv phage display

Electrocompetent XL-1 Blue cells were transformed with each ligated library and phagemids were rescued by the addition of the 2×10^{12} pfu of VCSM13 helper phage (Stratagene, La Jolla, CA) as described (Payne et al., 2005). Phage libraries were precipitated using PEG as previously described and stored in PBS containing 1% (w/v) BSA prior to panning (Payne et al., 2005). XL-1 Blue Electrocompetent cells were grown to an OD_{600} of 0.6. Phage library dilutions of 10^{-7}, 10^{-8} and 10^{-9} were incubated with the cells at room temperature for 15 min and plated on carbenicillin impregnated LB plates. Plates were incubated overnight at 37 °C and colony forming units were counted the next day to determine phage library titers.

2.9. scFv expression and Ag-specific binding

MaxiSorp ELISA plates (ThermoFisher Scientific, Rochester, NY) were coated with 1 μg of canine parvovirus (CPV) capsids for 5 min at 4 °C. Wells were blocked with 5% (w/v) nonfat dry milk in PBS for 1 h at 37 °C prior to the addition of the phage library. Plates were incubated with undiluted phage at 37 °C for at least 1 h. Unbound phage was removed by immediately washing seven times with 0.5% (v/v) Tween 20 in PBS. Bound phage was eluted using 0.1 M glycine–HCl (pH 2.2). Eluates were neutralized using 2 M Tris–base solution, then used to transform XL-1 Blue cells. Enriched phagemids were rescued using VCSM13 helper phage (Stratagene) and Ag-specific enriched phage was amplified overnight. Amplified phage was titered and used as the input phage for the next consecutive round of panning. Input and output (eluate) phage titers were determined as previously described (Barbas, 2001). After each panning, amplified eluted phage was analyzed for CPV-specific scFvs by phage ELISA.

2.10. Phage ELISA

1 μg of CPV capsid Ag and BSA (negative control) were incubated on MaxiSorp ELISA plates at 4 °C for 5 min. Plates were blocked with 5% milk in PBS for 1 h at 37 °C. Undiluted phage was incubated with plate bound Ag for 1 h at 37 °C. Plates were washed seven times with PBST. Bound phage was detected using a 1:5000 dilution of horseradish peroxidase (HRP) conjugated anti-M13 Ab (Abcam Inc., Cambridge, MA) in 5% milk. Plates were washed seven times and bound phage was detected with TMB (3,3′,5,5′-tetramethylbenzidine) peroxidase substrate (Thermo Scientific, USA). OD was read at 650 nm after 60 min using a Spectrafluor plus® spectrophotometer (Tecan US, Inc., Mannedorf, Switzerland).

3. Results

3.1. Primer design

The canine IgG-A nucleotide sequence (GenBank Accession no. AF354264) was used in a megablast search of the NIH GenBank database to identify and retrieve cloned and/or predicted immunoglobulin VH chain nucleotide sequences in the canine genome (Tang et al., 2001). Eighty-five nucleotide sequences were identified and aligned using the CLUSTALW multiple alignment program (EMBL, Heidelberg, Germany). Nucleotide sequences corresponding to the first 9 amino acids of the highly conserved VH FR1 sequence (EVQLVESGG—gag gtg cag ttg gtg gag tct ggg gga gac) were identified, aligned and assembled into 8 groups based on sequence homology. Eight degenerate primers were designed (designated Canine Single Chain Variable Heavy 1–6-Forward (CSCVH1–6F and CSCVH8–9-F)) that together would anneal to all identified VH FR1 nucleotide sequences.

To ensure that all known and predicted sequences of immunoglobulin VH regions would be amplified, the protein sequence of the canine IgG-A (GenBank Accession no. AAL35301.1) was used to BLAST search the NIH GenBank protein database to identify canine FR1 protein sequences that were not encoded by the previously identified nucleotide sequences. Sixty-four protein sequences were identified and aligned using the CLUSTALW program to identify highly conserved FR1 sequences. Eighteen dif-

ferent FR1 protein sequences were identified that were not encoded for by the nucleotide sequences previously identified. These protein sequences were back translated into nucleotide sequences using a standard mammalian codon usage table and a single degenerate oligonucleotide primer was designed (CSCVH7-F) that would anneal to unique nucleotide sequences that encode FR1 protein sequences. In order to amplify VH chains specifically from the Ag-experienced immunoglobulin repertoire, a single non-degenerate reverse primer was designed within the constant IgG region that encodes the highly conserved "STTAPSV" protein sequence. This primer was designated Canine Single Chain Gamma 1234-B (CSCG1234-B).

To design primers that would amplify canine VL lambda chains, predicted *Canis familiaris* immunoglobulin lambda chain nucleotide sequences from each of the four VL lambda chain families [V-I (GenBank Accession no. XM845300), V-II (GenBank Accession no. XM543519), V-III (GenBank Accession no. XM844188) and V-IV (GenBank Accession no. XM844237)] were used in a megaBLAST search of the *C. familiaris* NIH GenBank database using the default settings for non-redundant sequences http://blast.ncbi.nlm.nih.gov/Blast.cgi. Over 100 different VL lambda sequences were identified with the majority belonging to the V-I lambda chain family. Nucleotide sequences were aligned and unique sequences within FR1 of lambda chains were identified. Highly homologous sequences were grouped together and degenerate primers were designed to anneal to all sequences within each group. This resulted in the design of 12 degenerate forward primers (designated Canine Single Chain (CSC) CSCLam1a-dF and CSCLam2–9F).

Protein sequences that were not encoded by the above nucleotide sequences were identified following a BLAST search of the canine GenBank database using the VL lambda FR1 amino acid sequence (VLTQPAS). These protein sequences were back translated using the mammalian codon usage table and four degenerate forward primers (CSCLam10–13F) were designed that would collectively anneal to all unique nucleotide sequences that encode these VL lambda FR1 proteins. To design primers that would bind within the J region of canine VL lambda chains, a BLAST search of the canine genome was performed using the V–J region of the human lambda chain (GenBank Accession no. M36956). Predicted canine immunoglobulin lambda chain sequences were aligned and two degenerate primers were designed to anneal to 24 base pair sequences within the J segment (designated Canine Single Chain Joining (CSCJ) CSCJLam1-B and CSCJLam2-B). The location of the canine J segment was determined by sequence alignment with the J segment of the human lambda chain sequence (Gen Bank Accession no: M36956, nucleotides 1–33).

To design forward primers that anneal to canine VL kappa sequences, the predicted *C. familiaris* immunoglobulin kappa chain V-I (Gen Bank Accession no. XM849621), V-II (Gen Bank Accession no. XM844874), V-III (Gen Bank Accession no. XM849629) and V-IV (Gen Bank Accession no. XM849668) sequences were used in a megaBLAST search of the *C. familiaris* NIH GenBank database. All nucleotide sequences were aligned and grouped according to sequence homology within FR1. Two non-degenerate primers were designed to anneal to the V-I sequence (designated Canine Single Chain Kappa (CSCK) 1-F) and a V-II sequence, (designated CSCK6-F)). In addition 4 degenerate primers were designed to anneal to sequences within VII and V-IV (designated CSCK24-F), within V-III and V-IV families (designated CSCK34-F), within the V-IV family (designated CSCK4-F) and within the V-II and V-IV families (designated CSCK5-F). Reverse primers for canine kappa chains were designed within the J regions of the kappa chains that were identified by sequence alignment with human VL kappa chain J regions. All nucleotide sequences of canine VL kappa chains were aligned and sequences encoding the J region identified. Four degenerate primers were designed to anneal to all J regions and these primers were designated as Canine Single Chain Joining Kappa (CSCJK) 1B-4B. Nucleotide sequences of all primers designed are listed in Table 1.

To enable VH and VL amplicons to be spliced together to generate scFvs the 5′ region of all VH forward primers (CSCVH1–7F) and the 3′ end of the VL (lambda and kappa) reverse primers were designed to encode complementary overlapping sequences of a flexible glycine–serine linker. In addition, conserved recognition sites for the second round PCR primers (RSC-F and RSC-B) were incorporated into the 5′ end of the VL forward primers and the 3′ end of the VH reverse primer (see Table 1 for RSC-F and RSC-B sequences (Barbas, 2001)).

3.2. Amplification of VH and VL chains from canine splenocytes

First round PCR amplification of VH and VL chains was performed on cDNA derived from canine splenocytes. In all samples, PCR products of ~450 bp were amplified using each VH forward primer and the CSCG1234-B reverse primer (Fig. 1A). The quantity of product produced using the CSCVH7-F primer, designed on back translated protein sequences was substantially less than that obtained with the other VH forward primers (Fig. 1A). Amplicons of ~350 bp were produced with all forward and reverse λ primers designed on nucleotide and back translated protein sequences (Fig. 1B). Similarly, amplicons of ~350 bp were produced with all VL kappa primers except for the CSCK1-F primer (Fig. 1C). Use of CSCJK2-B in combination with forward kappa primers only produced a faint band with CSCK24-F, CSCK5-F and CSCK6-F. All VH and VL (lambda) and VL (kappa) amplicons were pooled, gel purified and VH chains were randomly combined with either λ or κ light chains in a second round PCR reaction to generate separate libraries of VH–VLλ and VH–VLκ scFv fragments of ~850 bp (Fig. 2).

Test ligations were performed to identify the optimum molar ratio of pComb3X vector:scFv insert to generate large and diverse libraries. The greatest number of transformants was produced using a 1:2 backbone:scFv insert molar ratio and this ratio was chosen for library ligation (data not shown). Following scFv library ligation and transformation, the number of transformants per microgram of vector DNA for both VH–VLλ and VH–VLκ scFv libraries was approximately 1×10^8 and between 3 and 5 ligations were

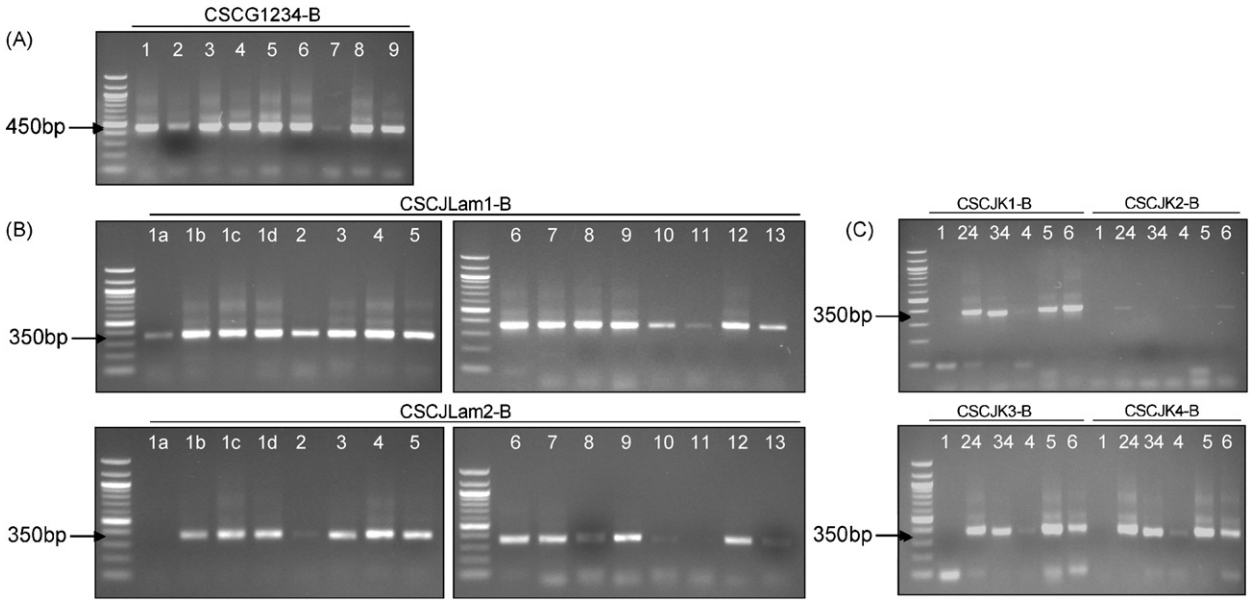


Fig. 1. VH and VL (lambda and kappa) chain PCR products generated from canine splenic cDNA. (A) VH amplicons generated using CSCVH1-F to CSCVH9-F forward primers (labeled 1 through 9) and CSCG1234-B reverse primer. (B) VL lambda amplicons generated using CSCLam1a-F to CSCLam13-F forward primers (labeled 1a through 13) and CSCJLam1-B reverse primer (upper panel) and CSCJLam2-B (lower panel). (C) VL kappa amplicons generated using CSCK1-F, 24-F, 34-F, 4-F, 5-F and 6-F forward primers (labeled 1, 24, 34 and 4 through 6) and CSCKJ1-B and CSCKJ2-B reverse primers (upper panel) and CSCKJ3-B and CSCKJ4-B reverse primers (lower panel). In all cases, 5 μl of each 100 μl PCR reaction were loaded onto a 1% agarose gel for analysis. A 100 bp ladder was used in each gel.

performed for each library to achieve an estimated library size of between 3 and 5×10^8.

3.3. Sequence analysis of canine scFv libraries

To determine library diversity, PCR-amplified scFv fragments from 20 individual clones of each VH–VLλ and VH–VLκ library were analyzed by BstNI fingerprinting (Fig. 3). Each scFv had a different digestion pattern indicating variations in BstNI recognition sites amongst individual scFvs and confirming diversity amongst randomly selected clones. To confirm that the scFvs consisted of VH and VL chains of canine immunoglobulin molecules and to further analyze library diversity, DNA sequence analysis was performed on 50 randomly selected clones obtained from a VH–VLλ and VH–VLκ library. All scFv sequences were analyzed using the web-based NCBI Ig BLAST program (http://www.ncbi.nlm.nih.gov/igblast/) to approximate framework and complementarity determining regions. In the 2 libraries sequenced, the majority of VH and VL (λ and κ) chains had open reading frames encoding full length VH and VL chains.

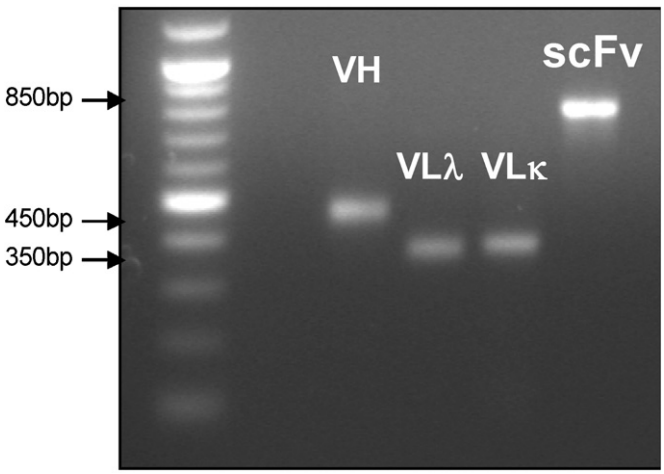


Fig. 2. Generation of canine scFv constructs. All canine VH amplicons (450 bp) and VL (lambda) and VL (kappa) (~350 bp) amplicons were pooled, gel purified and randomly combined to generate scFv constructs. scFv constructs (~850 bp) shown here represent a mixed library containing both lambda and kappa light chains.

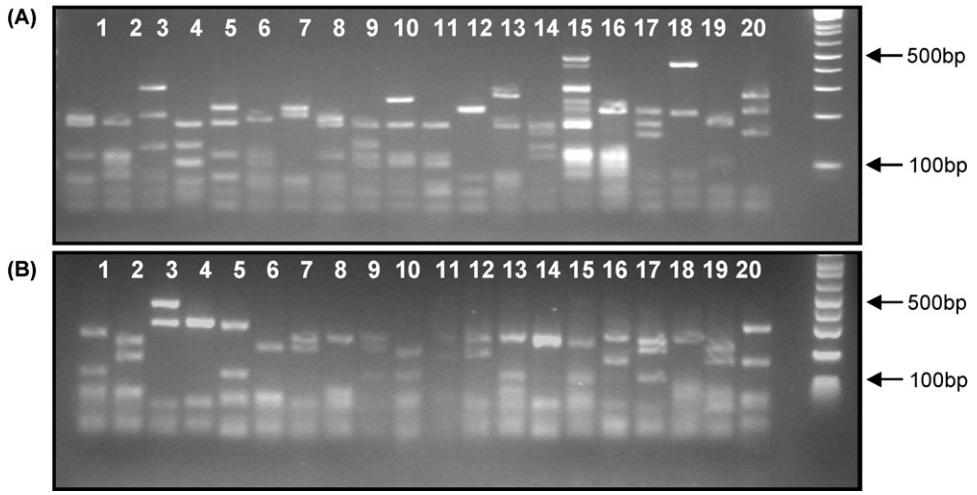


Fig. 3. BstNI fingerprinting of canine VH–VLλ and VH–VLκ scFv phage display libraries. Twenty scFv clones of (A) VH–VLλ and (B) VH–VLκ libraries (unpanned) were randomly selected and scFv inserts were amplified using pCOMB3X sequencing primers (5LC and DP seq). Amplified products (1–20) from each library were digested with the restriction enzyme BstNI and analyzed on a 3% agarose gel. A 100 bp ladder was used in each gel.

Analysis of randomly selected VH sequences from both scFv libraries revealed that the vast majority of heavy chains belonged to the V-I gene family (classified as mammalian clan III) (Table 2). These included 10 different V germline genes as determined by the human sequence subgroup program in the Kabat database. As reported in other species, the CDR3 of canine VH sequences showed the greatest variation in length. Within the sequenced clones from VH–VLλ libraries, the majority of VL chains belonged to the V-I lambda family. However, lambda chains belonging to the V-II, V-III and V-IV families were also identified indicating that the designed primers amplified members of all lambda families (Table 3). 10 different V germline genes were identified within the sequenced VL lambda chains. Within the sequenced clones from the VH–VLκ library, only VL chains belonging to V-II and V-III kappa families were identified (Table 4) which included 4 different kappa germline genes. Less variation in the length of CDR3 of VL sequences (λ and κ) was observed in comparison to VH chains (Tables 3 and 4).

3.4. scFv library validation

To determine whether Ag-specific scFvs could be identified and isolated from constructed libraries, we screened a canine scFv (VH–VLλ) phage display library for the presence of scFvs that bound to the well-characterized canine parvovirus (CPV) viral capsid. The panned library was generated from the splenocytes of a dog previously vaccinated against CPV and as such, scFvs that bind to the capsid should be present within the library. The VH–VLλ library was panned four times against immobilized CPV capsids. Input and output numbers of phage were determined before and after each panning procedure (Fig. 4A). Following each panning, the number of eluted phage steadily increased suggesting enrichment for Ag-binding scFvs. To confirm that the scFv phage library was progressively enriched for CPV-specific binders, amplified phage from each consecutive round of panning was evaluated for CPV-specificity by ELISA (Fig. 4B). Following each round of panning, phage libraries showed a steady increase in Ag-specific phage binding. In contrast, binding of panned phage libraries to BSA remained at background levels. To identify individual CPV-specific scFvs, scFv clones derived from eluted phage (4th pan) were screened for CPV-specificity by phage ELISA (Fig. 5A). Sequence analysis of two CPV-specific clones showed that both scFvs utilized a VH-I gene linked to a VLλ V-I gene (Fig. 5B). Blast analysis of these sequences against the human germline V genes in the Kabat database indicated that these sequences likely represent different alleles of the same germline VH gene. In comparison, the CPV-specific VL chains appeared to represent different germline VL genes. Taken together these results indicate that Ag-specific scFvs can be isolated from canine scFv phage display libraries generated using the primers described in this report by simple panning techniques.

4. Discussion

Although strategies to design degenerate primers that amplify the VH and VL genes of the mouse, human, rabbit, chicken, and other species-specific immunoglobulin repertoires have been described, there are no reports of similar approaches being employed to generate scFv libraries in the domestic dog (Chiang et al., 1989; Orlandi et al., 1989; Coloma et al., 1991; Wang et al., 2000). Here we describe the design and validation of a set of degenerate primers that amplify rearranged canine VH and VL chain genes and allow for the random combination of these chains via a flexible linker to form scFvs. In this study we chose to design the reverse VH primer within the constant IgG region such that the scFv libraries generated would encompass the antigen-experienced Ig repertoire of each individual dog. This strategy was employed to take advantage of the increased antigen affinity afforded by somatic hypermutation that occurs within germinal

Table 2
VH amino acid sequences of scFv clones generated from canine splenocytes.

Fri	CDRI	FR2	CDR2	FR3	CDR3	FR4	Constant
Heavy chain V-I							
EVQLVESGGDLVKPGGSLRLSCAASGFTFS	TYFMS	WVRQAPGKGLQWVA	RITEDGSSANYADAVRG	RFTISRDNAENTLYLQMNSLRADDTAVYYCVK	LVPGSYRLFYGVDY	WGPGTSLFVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFTFS	SYDMS	WVRQAPGKGLQWIT	AIKSDGTTTYYIDAVKG	RFTVSRDNARNTVYLQMNSLRAEDTAMYYCAR	DDIFMDRVGMDY	WGRGTSLFVSS	ASTTAPSV
EEQLVELGGDLVKPGGSLRLSCVASGFTFS	SYYMF	WVRQAPGKRLQWVA	DITYAGTVYYADITEG	RFTISRDNAKNTVYLQMASLTAEDTAVYYCTK	LGGSSAWGDY	WGPGTSVFVSS	ASTTAPSV
ELQLVELGGDLVKPGGSLTLSCVTSGFTFS	DYAMT	WVRQAPGKGLQWVA	YINTGGTTTYYADAVKG	RFTISRDDARNTLYLQMNSLRSEDTAVYYCLG	ATVAYFYGLDY	WGHGTSVFVSS	ASTTAPSV
EVQLVETGGGLAKPGGSLRLSCVASGLSFS	SYSMS	WVRQAPGKGLQWVT	AIDYHGRDTFYTDTVKG	RFTISRDDARNTMYLHMDGLRAEDTAVYYCMV	YGSHLTFDF	WGQGTLVTVSS	ASTTAPSV
EVKLVESGGDLAKPGGSLRLSCAASGLAFS	SHSMN	WVRQAPGKGLQWVT	AISYDGRRIYYSDDVKG	RFAVSRDNARNTMYLQMTGLTVADTGLYYCAI	VGLGWQLANFEF	WGQGAQVIVAS	ASTTAPSV
EVQLVEFGGDLVKPGGSLRLSCVASGFTFS	SYDMD	WVRQAPGKGLQWLS	EISSSGSSTYYADAVKG	RFTISRDNAKNTLYLHMNSLRPEDTAVYYCTK	GGVKAPYKSGVDY	WGPGTSVLVSS	ASTTAPSV
EVRLVESGGDLVKPGGSLRLSCVASGFTFS	DYDMD	WVRQAPGKGLQWLS	EINSSGSNTFYADAVRG	RFTVSRDNAKHTVYLQMNGLRAEDTAVYYCAR	GWGKNTFAPFDY	WGQGTLVTVSS	ASTTAPSV
EVQLLESGGDLVKPGGSLRLSCVASGLTFS	SHDMD	WVRQAPGKGLQWLT	RITNDGRSTDYADAVKG	RFTISRDNAKNTLYLQMNSLRAEDTALYYCAR	GGTMSPWY	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFSIG	DSDTN	WVRLAPGKRLQWVA	GISVDGISTYYIDAVKG	RFTISRDTAKRTVYLQMNSLRAEDTAVYYCGP	GSGYY	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFTLS	DSDTN	WVRLAPGKRLQWVA	GISVDGISTYYIDAVKG	RFTISRDTARKTVYLQMNSLGVEDTAVYFCSS	GYIYMDTIADN	WGQGTLVTVSP	ASTTAPSV
EVQLVEFGGDLVKPAGSLKLSCVASGFTFR	HYDIH	WVRQAPGRRLQYVA	GIHYDGSYIYYIDAVKG	RFTISRDNARNTVYLQMNSLRVEDTAVYYCVK	APGLEY	WGQGTLVTVSS	ASTTAPSV
GVQLVESGGDLVKPGGSLRLSCVVSGITIN	TYTYGMS	WVRLSPGRGLQSVA	HLSRAGYTYYADAVKG	RFTISRDNGKSTLYLQMNSLTVEDTAVYYCVK	APLRSGGVDY	WGQGTLVTVSS	ASTTAPSV
DVQLVESGGDLVRPGGSLTLSCVASGFTFT	NYDMY	WVRQPPGKGLEWVA	RIYETGSTTYYAEVVKG	RFTMSRDNAKSMAYLQMNSLRAEDTAVYYC	ANLTPQRDIYGPGDY	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGESLRLSCVASTFTLR	KYDVY	WVRQVPGAGLEWVA	RISDSGSTTFYAEYVEG	RFTITRDNGKNMAFLQMNSLRAEDTALYYCA	ISLSWRWGFDY	WGQGTLVTVSS	ASTTAPSV
EVQLLESGGDLVRPGESLRLSCIASGFTFN	TYTMA	WVRQGPGKGLEWVA	GISSDGSSPYHSAAVKG	RFTISRDSARSTVYLQMNSLRAEDTAVYYCAK	DALSSWGPHNFDH	WGQGTLVTVSS	ASTTAPSV
EVQLVQSGGDLVKPGGSLRLSCVASGFTFS	RYRMA	WVRQPPGRGLQWVA	FINSDGDRTTYSDTVKG	RFTISRDNANDTLYLQMNSLRDEDTALYFCAS	DALYGTSWYSILDY	WGQGTLVTVSS	ASTTAPSV
EVSLVESGGDLVKPAGSLRLSCVASGFTFS	DYDMY	WVRQAPGKGLQWVA	VISYGGIDTYSDTVKG	RFTISRDNARNTVYLQMNSLRAEDTAVYFCAR	SSFRTDLNY	WGQGTLVIVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFTFS	NYDMT	WVRQAPGKGLQWVA	AVSFVGGSTYYTDALKG	RFTISRDNARNTVYLQMNDLRAEDTAVYFCAD	NTYNWGWGAEY	WGQGTLVTVSS	ASTTAPSV
EVPLVESGGLVKPGGFLRLSCVASGFTFS	NSTMT	WLRQAPGKGLQLVA	YISYGGGTTYYVEDVKG	RFTISRDNAKNTLSLQMDSLRAEDTAVYFCAA	QSSSGWGYFVSEY	WGQGTLVTVSS	ASTTAPSV
ELQLVELGGNLVKPGGSLRLSCVASGSTFK	NYYMD	WVRQAPGKTLEWVA	GISSDGYKTYYGQAVQG	RFTISRDNAKNTLYLQMDGLTVEDSAVYYCAM	EGGVHSESWFADFDS	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGESLRLSCVASGFILR	KYYLH	WVRQAPGKGPQWVA	RISGEGYKTYYADAVRG	RFTISRDNAKSTIYLQMDTLTAEDAGIYYCVK	DSDAPLHS	WGDGTLVAVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFIFS	SYYIY	WVRQSPGKGLQWVA	RINNDGSRIYYADAVKG	RFTISRDIAKDTLYLQLDRLGAEDTAVYYCVP	PRVHVWLGDFDS	WGQGTLVTVSS	ASTTAPSV
DVQLVESGGDLVKPGGSLRLSCVASGITLS	NYYMC	WVRQAPGKGLQWVA	RISYDGGITEYADAVKG	RFTISRDNAKNTLYLQMNSLRADDTAMYYCTQ	GIDGPY	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFTFG	DYYMN	WVRQAPGKGLQWVA	YIHSGGGSTTYADAVKG	RFTISRDNAKNTLYLQMNGLRAEDTALYYCAS	GSLGTYGRYYSFDY	WGQGTLVTVSS	ASTTAPSV
GVQLVESGGDVVKPGGSLRLSCVASGFTFS	SYYMY	WARQAPGKGLQWVS	HIKRDGSSTSYADAVKG	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAK	DLGTYGYNLEY	WGRGTLVTVSS	ASTTAPSV
EVQLLETGGDLVKPGESLRLSCVASGFSFS	NYYMT	WVRQAPGKGLQWVG	YIDNDGSGTDYADSVKG	RFTISRDNARNTLYLQMTSLRVEDTAVYYCAS	DLWGHYGSYRGPRLLLDN	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPGGSLRLSCVGSGFTFS	TYVMT	WVRQAPGKGPQWVA	SINSGGTSATYADAVKG	RFTISRDNAKNTLYLQINSLRAEDTAVYHCAG	LYMYSPSRALEF	WGQGTLVTVSS	ASTTAPSV
EVQLVETGGDLVKPGGSLRISCVASGFDFH	SSHMT	WVRQTPGKGLQWVA	SINSGRGTGYADAVKG	RFTISRDNAKNTVYLRMNSLTAKDTAVYYCA	GRIAVES	WGQGTLVTVSS	ASTTAPSV
GVQLVESGGALVKPAGSLRLSCVTSGFIFT	YYGMS	WVRQAPGKGLQWVA	HIYSDGSGTTYADAVKG	RFTISRDNAKNTVHLQMNSLRAEDTAVYYCAR	DMDWGAWVDLEY	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPAGSLTLSCVASGITFS	KYDVI	WVRLAPGKGLQWVA	GISNNGNTAYADAVVG	RFTTSRDIAKNTVYLRMNSLTAEDTAVYYCVA	GLKY	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPAGSLRLSCVASGFTFS	SYDMS	WVRQAPGKGLQWVA	GIMADGSTYYADAVKG	RFTISRDNAKNTVYLQMDSLRAEDTAMYYCAK	DRLSYYIYCLEY	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPAGSLRLSCVASGFTFS	TYGMD	WVRQAPGKGPQWVG	HIRTSGDTRYADAVKG	RFTISRDNAKNTVYLQMDSLTVEDTAFYFCAK	DGLRYGYVPDFEH	WGQGTLVTVSL	ASTTAPSV
EGQLAESGGDLVKPAGSLRLSCVASGFTFS	DYDMS	WVRQAPGKGLQWVA	GISYDGRSTYYTDAVKG	RFTVSRDNARNTLFLQMNSLRAEDTAMYYCAK	VDWFTRNWYIDF	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPAGSLRLSCVASGFTFS	DYDMS	WVRQAPGKGLQWVA	GISYDGRSTYYTDAVKG	RFTVSRDNARNTLFLQMNSLRAEDTAMYYCAK	VDWFTRNWYIDF	WGQGTLVTVSS	ASTTAPSV
EGQLAESGGDLVKPAGSLRLSCVASGFTIS	SHDMS	WVRQAPGKGLQWVA	GINSGGTRTGYTDAVKA	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCVL	SIVTTN	WGRGTLVTVSS	ASTTAPSV
GVQLVESGGDRVNPAGSLRLSCVASGFTFS	SHDMN	WVRQAPGKGLQWVA	SINSGGSGLHYADSVRG	RFTVSRDNAKNTLYLDLNDVRDEDTAMYYCTT	EKLGYHNPFGFWD	WGQGTLVTVSS	ASTTAPSV
EVQLVQSGGDLVKPGGSLRLSCVASGFTLS	NFDMQ	WVRQAPGKGLQWVA	YINSGANTTYYADAVRG	RFTVSRDNAKNTLYLQMNSLTAEDTAVYYCTD	RGGH	WGQGTLVTVSS	ASTTAPSV
EVRLVESGGDLVKPAGSLRLSCVASGFTFS	SYTMS	WVRQAPGKGLQLVA	NIKSGGTYTYYTDAVQG	RFTISRDNAKNTVYLQMNSLRAEDTAMYYCTK	EGYYDTFFDC	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPAGSLRLSCVASGFTFS	SYSMS	WVRQAPGKGLQLVA	GIISGGSSTYYTDAVKG	RFTVSRDNAKNTVFLQMNSLRAEDTAMYYCAK	EKYTYGYGAGLEY	WGQGTLVTVSS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFAFS	SYGMS	WVRQSPGKGLQWVA	DIRSTGDTYYADAVKG	RFTISRDNAENTLYLQMSSLRVEDTAIYYCAS	GPVDTTEH	WGQGTLVTASS	ASTTAPSV
EVQLVESGGDLVKPGGSLRLSCVASGFTFS	DYGMI	WVRQSPGKGLQWVA	ALSSSGSSTYYADAVKG	RFTISRDNAENTLHLQMKSLRAEDTAVYYCAK	GFGGLYIRMDNIEY	WGQGTLVAVSS	ASTTAPSV
ELQLVEFGGDLVKPGGSLRLSCVASGFTFS	SYGMT	WVRQSPGKGLQWVA	DISSSGTTYHADAVKG	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAK	QVQGSLPPDH	WGQGTLVTVSS	ASTTAPSV
EEQLVELGGDLVKPGGSLRLSCVASESTFG	VFVMT	WVRQSPGKGLQWVA	DYSSTGSTYYMDAVRG	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAS	PQEMGLFTAS	WGQGTLVTVSS	ASTTAPSV
DVQLVESGGDLVKPGGSLRLSCVASGFTFS	NYGMS	WVRQSPGKGLQSVA	VINNGGDYIHYTGAVKG	RFTISRDNAKNTLYLQMNSLRAEDTAVYYCAK	ATILGFGHES	WGQGTLVTVSS	ASTTAPSV

Table 3
VL lambda amino acid sequences of scFv clones generated from canine splenocytes.

FRI	CDRI	FR2	CDR2	FR3	CDR3	FR4
Lambda V-I						
ALTQQASVTGSLGQTVTISC	TGSSSNIGGYNVG	WFQQVPGTGPRTVIY	SSTDRPS	GVPDRFSGSRSGNTATLTISGLQAEDEAVYYC	STWDRGLKSGV	FGGGTHLTVL
VLTQLASVSGSLGQRVTISC	TGSSSDVGYGDYVG	WYQQLLGRSPRTLIY	DTTSRPS	GVPDRFSASRSGNTATLTISGLQAEDEGDYYC	SSYDNSLSGTV	FGGGTHLTVL
VLTQLASVSGSLGXRVTISC	TGSSSNVGYGNYVG	WYQQLPGASPRTLIY	DSSSRPS	GVPDRFSGSRSGSTATLTISGLEAEDEADYYC	SSFDNSLHGTV	FGGGTHLTVL
VLTQLTSVSGSLGQRVTISC	TGSRSNVGYGNHVG	WYQQFPGTGPRTLIY	FSDSRPS	GVPGRFFAIRSGSTATLTISGLQAEDEADYYC	SSFDNDLRGHV	FGGGTQLTVL
VLTQLPSVSGSPGQMVTISC	TGSSSNVGHGNYVG	WYHQVPGTGPKTLIY	YSGGRFS	GVPDRFSDSRSDSTATLTISGLQADDEADYYC	SSYAGTDTFV	FGGGTQLTVL
VLTQLASVSGSLGQEVTISC	TGSSSNIGRAYVG	WYQQFPGSGPKTLIY	GNNNRAS	GVPDRFSGSRSGNTATLTISGLQAEDEADYYC	SSWDYSLSAV	FGGGTHLTVL
VLTQLASVSGSLGQEVTISC	TGSSSNIGRAYVG	WYQQFPGSGPKTLIY	GNNNRAS	GVPDRFSGSRSGNTATLTISGLQAEDEADYYC	SSWDYSLSAV	FGGGTHLTVL
VLTQLASVSGSLGQRVFISC	TGNTSNIGRGYVG	WYQQLPGTGPRTLIY	GLSNLSP	GVPNRFFGSRSGNTATLTIAELQAEDEADYYC	SSWDRSVSTPL	FGGGTQLTVL
VLTQLPSVSGSXGQKVTISC	TGSSSNIXANYVG	WYQQFPGMGPKTVIY	VNDHRPS	GVPDRFSGSKSGSSATLTISGLQAEDEADYYC	SSWDDSFRHTV	FGGGTQLTVL
VLTQLASVSGSLGQKVTISC	TGSSSNIGANYVG	WYQQFPGMGPKTVIY	VNDHRPS	GVPDRFSGSKSGSSATLTISGLQAEDEADYYC	SSWDDSLRHTV	FGGGTHLTVL
VLTQPASVSGSLGXRITISR	TGGSSNIGSNNVN	WYQQLPGRGPRIVIY	FRNSRPS	GVPDRFSGSKSGSTATLTISGLQAEDEADYYC	STWDDSLTAVV	FGGGTQLTVL
LLTQPASVSGSLGXXITISC	TGSSSNIGGNNAA	WYQQLPGRGPRTVIY	GINSRPS	GVPDRFSASKSGSTATLIISGLQAEDEADYHC	STWDDSLRGFL	FGGGTQLTVL
VLTQLTSVSGSLGQRITISC	TGSSSNIGGNNVY	WYQQLPGRGPRTVIY	GTKISS	GVPDRFSGSKSGSTSTLTISGLQAEDEADYHC	SAWDDSLNAPV	FGGGTQLTVL
LLTQPASLSGSLGXRITISC	AGSNSNIGTNNVN	WYQQVPGRGPRTVIY	FTNRRPS	GVADRFSGSKSGTTATLTISGLQAEDEADYYC	STWDDSLPGVV	FGGGTHLTVL
VLTQLASLSGSLGQRITISC	AGSNSNIGTNNVN	WYQQVPGRGPRTVIY	FTNRRPS	GVADRFSGSKSGTTATLTISGLQAEDEADYYC	SSWDSSLSAIV	FGGGTHLTVL
VVTQAASVSGSLGLRVTISC	TGSSSDIRRDYVA	WYQQLPGTGPRTLIY	GNNNRPS	GVPDRFSGSRSGTTATLTISGLQTEDEADYYC	ASWDFSLYGPV	FGGGTHLTVL
VLTQPASVSGSLGQRVTISC	TGSRSNIGRDYVA	WYQEFPGTGPRTLIY	GNNNRVL	GVSDRFSASRSGTTATLTISGVQTEDEADYYC	ASWEWSFYGPV	FGGGTHLTVL
ALTQLASVSGSLGQRVTISC	TGAGSNIDRDYVNWY	WYQQVPGTRPRTLIY	GSSNRPS	GVPARFSASKSGSTATLTISGLQAEDEADYYC	QSFEDTLGIV	FGGGTQLTVL
VLTQPPSVSAALGSSVTISC	TGTDTNIGNDYDLQ	WYQQFPGKSPKIIIF	GNVDRPS	GVPIRFSGSKSGTTATLTISGIQAEDGADYYC	QSYDDNLDGAV	FGGGTHLTVL
VLTQLASVSAALGQRVTISC	TGSNTNIGSGYDVQ	WYQQLPGKSPKTIIY	GNTNRLS	GVPVRFSGSKSGSTATLTITGLQAEDEADYYC	QSYDDNLDGAV	FGGGTHLTVL
PSSRKPASVSGSLEGFTISC	TGSRSNIGSDYVA	WYQQFPGTGPKTLIY	STTERPP	GVSDRFSGSRSGSTATLTISGLRAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
SRCIRRPQCLGPGKGVTISC	TGSRSNIGSDYVA	WYQQFPGTGPKTLIY	STTERPP	GVSDRFSGSRSGSTATLTISGLQAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
PARLCPAAMLGPLDRKYHLL	TGADPTSGSDYVA	WYQQFPGTGPKTLIY	STTERP	GVSDRFSGSRSGSTATLTISGLQAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
RVDSAGLSVWVPGKKVTISC	TGSRSNIGSDYVA	WYQQFPGTGPKTLIY	STTERPP	GVSDRFSGSRSGSTATLTISGLQAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
RADSAGLSVWVPGKKVTISC	TGSRSNIGSDYVA	WYQQFPGTGPKTLIY	STTERPP	GVSDRFSGSRSGSTATLTISGLQAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
ALTQLASVSGSLGQKVTISC	TGSRSNIGSDYVA	WYQQFPGTGPKTLIY	STTERPP	GVSDRFSGSRSGSTATLTISGLQAEDEADYYC	SAYDNSLSVTV	FGGGTHLTVL
ALTQPSSASGTLGQTVTISC	DGSSSNIGTSNYID	WYQQFPGTSPKLLLY	FTDKRPS	GIPARFSGSKYGNTAFLTISGLQAEDEADYYC	SSYAGTDTFV	FGGGTQLTVL
RPDSTFLGVWDFGQTVTISC	DGSSSNIGTSNYID	WYQQFPGTSPKLLLY	FTDKRPS	GIPARFSGSKYGNTAFLTISGLQAEDEADYYC	SSYAGTDTFV	FGGGTHLTVL
VLTQLTSVSGSLGQRVTISC	SGSANNFXIFGAS	WYRQLPGQAPKLLVY	SNGNRPS	GVPDRFSGSNSANSATLTISGLQAEDEADYYC	QSFDTTLDAAV	FGGGTHLTVL
VLTQLASVSGSLGQTVTISC	SGATNDIXIVGAS	WYQQLPGKAPKLLVY	SYGERPS	GVPDRFSRSKTGNSATLIITGLQAEDEADYYC	QSFDTTYHTAM	FGGGTQLTVL
VLTQPPSVSGSLGQTVTISC	SGRTNNIGSVGAA	WYQQLPGKAPKLLVF	NVGDRPT	GVPDRFSGSNSGNSATLTITGLQAEDEADYYC	QSFDTTLVV	FGGGTHLTIL
PADSACLSVGVPWKTVTISC	SGRTNNIGSVGAA	WYQQLPGKAPKLLVF	NVGDRAT	GVPDRFSGSNSGNSATLTITGLQAEDEADYYC	QSFDTTLVV	FGGGTHLTVL
RADSASFSVGVPGQTVTISC	SGRTDVDRFGTS	WYQQLPGKAPKLLVD	TDGNRPS	GVPDRFSGSKSGNSATLTITGLHAEDEADYYC	LSVGPKVNTHAV	FGGGTHLTVL
RADSAAVCVCSPGKRVTISC	TGSSSNIGRYSVG	WFQQFPGKGPRTVIQ	NTSNRPS	GVPDRFSGSESGSTAILTISGLQAEDEADYYC	STYDSSLGFV	FGGGTHLTVL
RADSASLSVWVPGKKVTISC	TGSSSNVGYGNYVG	WYQQLPGTSPRILIY	YSSSRPS	GVPDRFSGSRSGSTATLTISGLQAEDEADYYC	STWDNSVTYV	FGSGIQLTVL
RADSACLSVWVPGKRVTISC	TGSTSNIGSGMYVG	WYQQFPGTSPRTLIY	YGTSRPS	GVPDRFSGSSSGSTATLTISGLQAEDEADYYC	SSYDGFNNL	FGGGTHLTVL
RADSAGLSVWVPGYRVTISC	TGNSSNFGYDNYVA	WYQQLPGTSPRTLIY	ETGRRPS	GVPYRFSGSRSGNTATLTISGLQAEDEADYYC	SSYDNGLSVV	FGGGTHLTVL
Lambda V-II						
GLNQPSSVSGTLGQTVTISC	DGSSSNIGSHNWIE	WYQQFPGTSPKLLIY	YTNNRPS	GIPARFSGSKSGNTASLTISGLQAEDEADYYC	SAFAGSNNAAL	FGGGTQLTVL
ALTQPSSVSGTLGQTVTISC	DGSSSDIGSYSYIA	WYQQFPGTSPKLLIQ	YTDNRPS	GIPTRFSGSKSGNTASLTIPGLQAEDEADYYC	CAYAGSDTYV	FGSGTQLTVL
Lambda V-III						
GLTQLPSMSVALRQTARITC	GGGNIESKNVH	WYQQKLGQAPIQIVY	YDTRRPV	GIPERFSGAKSGNTATLTISGALAEDEADYYC	QVWDSGTLI	FGGGTQLTVL
GLTQLPSVNVTLRQTAHITC	GGDSIGSKYVQ	WIQQNPGQAPVMIIY	KDTNRPT	GIPERFSGANSGNTATLTISGALAEDEADYYC	QVWDSNTKRIV	FGGGTHLTVL
Lambda V-IV						
GLNQPPSVSVSLGQTATISC	SGESLSKYYAQ	WFQQKAGQAPVLVIY	KDTERPS	GIPDRFSGSSSGNTHTLTISGARAEDEADYYC	ESAVSTDTAM	FGGGTQLTVL
VLTQPPSVSVSLGQTATISC	SGESLSKYYAQ	WFQQRAGQVPVLVIY	KDTERPS	GIPDRFSGSSSGNTHTLTISGARAEDEADYYC	ESEVSTGTAV	FGGGTHLTVL
GLTQLPSVNVTLRQTAHITC	GGDRIGSKYVQ	WTQQNPGQAPVVIIY	KDTNRPR	GIPERFSGANSGNTATLTISGALAEDEADYYC	QVWDTSAKAV	FGGGTHLTVL
GLTQPPSVNVTLRQTAHITC	GGDSIGSKYVQ	WIQQSPGQAPLLIIY	KDSHRAT	GIPERFSAANSGSTATLTIAGALAEDEADYYC	QVWDNSVIA	FGGGTHLTVL

Table 4
VL kappa amino acid sequences of scFv clones generated from canine splenocytes.

FRI	CDRI	FR2	CDR2	FR3	CDR3	FR4
Kappa V-II						
MTQTPLSLSVSPGEPASISC	KASQGLLHSNGNTYLS	WFRQKAGQSPQRLIY	KVSIRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGLYYC	GQGIQDPWT	FGAGTKVDLK
MTQTPLSLSVSPGEPASISC	KASQSLLHSNGNTYLY	WFRQKPGQSPQRLIY	KVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGIYYC	GQGIQGPRT	FGAGTKVDLK
MTQTPLSLSVSPGEPASISC	KASQSLLHSNGNTYLY	WFRQKPGQSPQRLIY	KVSNRGP	GVPDRFSGSGSGTDFTLTISRVEADDAGVYYC	GQATQDPT	FGAGTKVDLK
MTQTPLSLSVSPGEPASISC	KASQSLLHSNGNTYLF	WFRQKAGQSPQRLIQ	KVSNRDP	GVPDRFSGSGSGTDFTLRISSVEADDAGVYYC	GQGTHNPPT	FGAGTKVEIK
MMQTPLSLSVSPGEPASISC	KASQSLLHSNGNTYLS	WFQQKAGQSPQRLIY	KVSNRDP	GVPDRFSGSGSGTEFTLRLSRVEADDAGVYYC	GQDTQDPFT	FGPGTKVEIK
MMQAPLSLSVSPGEPASISC	KASQSLLHSNGNTYLY	WFRQKPGQSPQRLIY	HVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGIYYC	GQVIQDPPT	FGAGTQVELK
MMQTPLSLSVSLGEPASISC	KASQSLLHSNGNTYFY	WFRQKPGQSPQRLIY	KVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDVGVYYC	GQGTQDPPT	FGAGTQVELK
MMQTPLSLSVSPGEPASISC	KASQSLLHSNGNTYLS	WFRQKPGQSPQRLIS	GVSKRDP	GVPDRFSGSGSGTDFILRISRVEADDGGIYYC	GQTTRYPLT	FGAGTKVELK
MMQTPLSLSVSLGETASISC	KASQSLLYSNGNTYLS	WFRQKPGQSPQRLIN	LVSNRDP	GVPGRFSGCGSGTDFTLRISRVEADDAGVYYC	GSLQAPRT	FGAGTQVELK
MMQTPLSLSVSLGETASISC	KASQSLLHSNGNTYLF	WFRQKPGQSPQRLIN	LVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGVYYC	GQGIQAPWT	FGAGTKVELK
MMQTPLSLSVSLGEPASISC	KASQSLLHSNGNTYLS	WFRQKPGQSPQRLIN	LVSNRDP	GVPGRFSGSGSGTDFTLRISRVEAEDVGVYYC	GQGTQDPPT	FGAGTKVELK
MMQTPLSLSVSPXXTASISC	KASQSLLHSNGKIYLF	WFRQKPGQSPQRLIN	LVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGVYYC	GQGTQEPWT	FGAGTKVDLK
MMQAPLSLSVSPGETASMSC	KASQSLLHSNGNTYLF	WFRQKPGQSPQRLIN	LVSNRDP	GVPDRFSDSGSGTDFTLRISRVEADDAGVYYC	GQGIQVPYT	FSQGTKLEIK
MMQAPLSLSVTFGDTASISC	KASQSLVYVNGKTYLF	WFRQRPGQSPQRLIN	LVSSRDP	GVPDRFSGSGSGTDFTLRISRVEADDTGVYYC	GQGIQFPYT	FSQGTKLEIK
MIQTPLSLSVSPGETASISC	KASQTLLYSNGNTYLF	WFRQKPGQSPQRLIN	LVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDTGIYYC	GQGIQSPYT	FSQGTKLEIK
MXQTPLSLSVSFGETASISC	KASQSLLHSNGNTYLF	WFRQKPGQSPQRLIN	LVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGVYYC	GQGIQAPT	FGQGTKLEIK
PSSCRRHCPVRQGETASISC	KASQSLLYSDGNTYLY	WFRQRPGQSPQRLIY	GVSKRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGVYFC	GQGIQDPYT	FSQGTKVEIK
SCRLHCPCPSALLETASISC	KASQSLLHSNGNTYLF	WFRQKAGQSPQRLIN	FVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDAGLYYC	GQGIYTPPT	FGAGTKLEIK
MMQTPLSLSVSPGETASISC	KASQSLLSSDGNTYLN	WFRQKPGQSPQRLIY	QVSNRDS	GVPDRFSGSGSGTDFTLRISRVEADDTGIYYC	GQVKQGPYT	FSQGTKLEIK
MMQTPLSLSVSPGETASISC	KASQSLLSSDGNTYLN	WFRQKPGQSPQRLIY	QVSNRDS	GVPDRFSGSGSGTDFTLRISRVEADDTGIYYC	GQVKQGPYT	FSQGTKLEIK
MTQTPLSLSVSPGETATISC	KASQSLLHSDGNTYLN	WFRQKPGQSPQRLIY	KVSNRDS	GVPDRFSGSGSGTDFTLRISAVEADGVGVYYC	GQGIQAPYT	FSQGTKLEIK
MMQAPLSLSVSPGETASMSC	KASQSLLHSNGNTYLY	WFRQKPGQSPQQLIY	RVSNRSP	GVPDRFSGSGSGTDFSLRISRVEADDTGVYYC	FQWTQDPCT	FGAGTKLEIK
MTQTPLSLSVSPGEPASISC	KASQSLLHSNGHTYLF	WFRHKPGQSPQSLIY	EVSNRDP	GVPDRFSGSGSGTDFTLRISRVEANDTGVYYC	GHGKEFPPT	FGAGTKVELK
MTQTPLSLSVSPGEPASISC	KASQSLLHSSGNTYLF	WFRHKSGQSPQRLIY	KVSNRDL	GVPDRFSGSGSGTDFTLRISRVEANDTGVYYC	RQGTQFPPT	FGAGTKVDLK
MTQTPLSLSVSPGETASISC	KASQSLLHSDGNTYLN	WFRQKPGQSPQRLIY	KVSNRDS	GVPDRFSGSGSGTDFTLRISRVEADDTGVYYC	MQGTEVPRT	FGAGTKVDLK
MMQAPPSLSLSLGEPASISC	KASQSLLSSNGNTYLY	WFRQKPGQSPEGLIY	KVSNRFT	GVSDRFSGSGSGTDFTLRISRVEADDAGVYYC	GQNLQFPST	FGAGTKVDLK
MTQTPPSLSVSPGEPASISC	RASQSLLSSNGNTYLT	WYLQKPGQSPQPLIY	LVSNRFT	GVSDRFSGSGSGTDFTLRISRVEADDTGIYYC	GQGTQYPRT	FGAGTKVDLK
HDADSTVPIRQVGEAASISC	KASQSLLSSDGNTYLI	WFRQKAGQSPQRLID	LVSNRDA	GVPDRFSGSGSGTDFTLRISRVEADDVGIYYC	GQGIQPWT	FGVGTKLEIK
DDADSTVPVRQLGEPASISC	KASQSLLHSDGDTYLF	WFQQKPGQSPRRLIY	RVSNRDP	GVPDRFSGSGSGTDFTLRISRVEADDVGIYYC	GQGIQDPLT	FGAGTKLEIK
DDADSTDPVRQLGETASISC	KASQSLLHSNGNTYLF	WFRQKPGQSPQRLIN	LVSSRDP	GVPDRFSGSGSGTDFSLRISRVETDDAGVYYC	GQALKAPNT	FSPGTKVEIK
HDTDPTVPVRSPGETASISC	KASRSLLHSDGNTYLF	WLRQRPGQSPQRLIG	LVSNRDP	GVPDKFSGSGSGTEFTLRISGVEADDVGLYYC	GQGTQDPWT	FGAGTQVELK
HDTDPTVPVRSPGETASISC	KASQSLLQSDGNTYLN	WFRQKPGQSPQRLIS	LVSNRDP	GVPDRFSGSGSGTDFTLTISRVEADDTGVYYC	MQGTQFPQT	FGAGTQVELK
HDTDPTVPVRSPGEPASISC	KASQSLLHSDGNTYLY	WFRQKPGQSPQRLIY	KVSNRDP	GVPDRFSGSGSGTDFTLRVSRVEADDAGLYYC	GQHIQYPLT	FGAGTQVELK
HDTDPTVPVRCPGETASISC	RASQSLLYSDGNTYLN	WFRQKPGQSPQRLIY	WVSNRDP	GVPDRFSGSGSGTDFTLRINRVEADDTGVYYC	MQGTQFPFT	FGQGTKLEIK
HDTDPTVPVRSPGEPASISC	KASQSLVHSNGNTYLY	WFRQRPGQSPQPLIY	QVSNRHP	GVPDRFGGSGSGTDFTLRISRLEADDAGVYYC	GQGTQDPPT	FGAGTKVELK
HDTDPTVSVLSPGTTASISC	KATQSLLHSNGDTELN	WFRQKPGQSPQALIY	KVSNRRP	GVPDRFSGSGSGTDFTLRISRVEADDAGVYYC	GHGIQTPRT	FGAGTKVDLK
DDAGSTVPGRQSGDLAIISC	RASQSLLRSDGKSYLN	WYVQKPGQTPRPLIY	EASKRFS	GVSDRFTGSGSGTDFTLKISRVEAEDVGVYYC	QQSLHFPWT	FGAGTQVELK
MMQTPLSLAVALGELATISC	RASQSLLHSNGKSYLN	WYLQKPGQTPRPLIY	ETSERLS	GVSDRFSGSGSGTDFTLEISRVEAEDVGIYYC	QQGLHFPPT	FGAGTKVDLK
Kappa V-III						
VMTQSPASLSLSQEEKVTITC	RASQSVSSYLA	WYQQKPGQAPKLLIY	GTSNRAT	GVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QQYNSGWT	FGAGTKVDLK
VMTQSPASLSLSQEEKVTITC	RASQSVSSYLA	WYQQKPGQAPKLLIY	GTSNRAT	GVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QQYNSGYT	FGQGTKVELK
VMTQSPASLSLSQEEEVTITC	RASQSVSSYLA	WYQQKPGQAPKLLIY	GTSNRAT	GVPSRFSGSGSGTDFSFTISSLEPEDVAVYYC	QHYNGGWT	FGAGTKVELK
VMTQSPASLSLSQDEKVTITC	RASQSISSYLA	WYQQKPGQAPKLLIY	GTSNRAT	GVPSRFSGSGSGTDFSLTISTLEPEDVAVYYC	QQYYIWWT	FGAGTQVELK
VMTQSPASLSLSREEEVTITC	RASQTITNSLA	WYQQKPGQAPKLLIY	ATSNRAT	GVPSRFSGSGSGTDFRFTISSLEPEDVGVYYC	QQYNSGWT	FGAGTKVEIK
VMTQSPASLSLSQEEKVTITC	RASQSLNNYLA	WYQQKPGQAPKLLIY	DTFKRAT	GVPSRFSGSGSGAEFTFTISSLEPEDVAVYYC	QQYYDGCT	FGPGTKVDLK

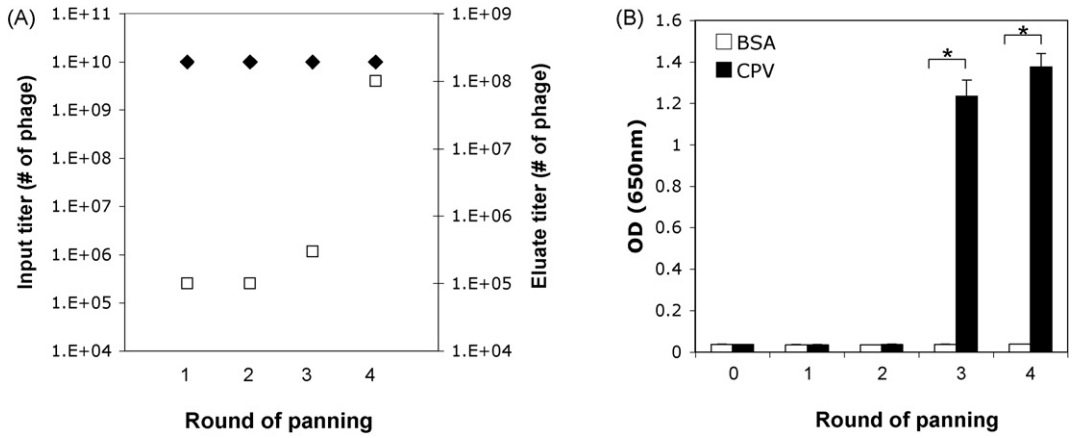
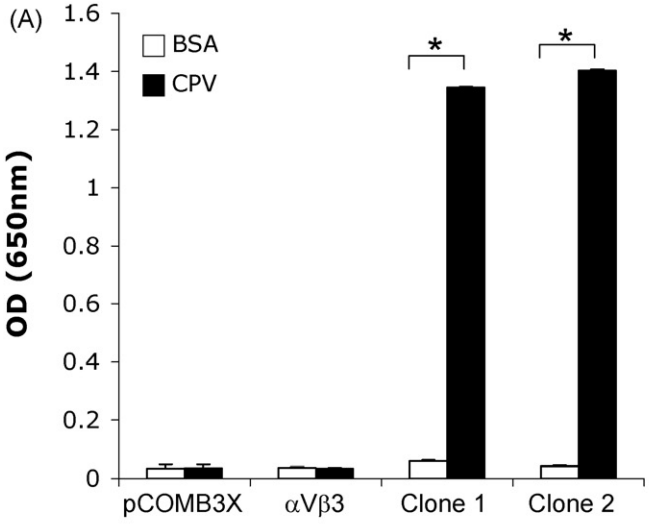


Fig. 4. Selection of CPV-specific scFv phage by panning on immobilized antigen. (A) A VH–VLλ scFv library underwent four rounds of panning against immobilized canine parvovirus (CPV) capsid antigen. CPV-bound phage was eluted and titered prior to amplification in order to determine the number of phage bound to CPV in each round of panning ("Eluate titer (# of phage)"; white squares). Phage titers of eluted phage that had been amplified overnight were determined and used to calculate the number of phage added into the next round of panning ("Input titer (# of phage)"; black diamonds). (B) Amplified phage from each consecutive round of panning was evaluated for CPV binding using a CPV-specific ELISA. Bovine serum albumin (BSA) was used as a negative control antigen. Statistical analysis was performed using a two-tailed Student's *T*-test where $^{*}p < 0.0005$.

centers during maturation of the antibody response. As such, scFvs generated from the antigen-experienced canine repertoire are more likely to have a higher affinity for a particular antigen than their IgM based counterparts. The negative side of this approach is that only scFvs with specificity for previously encountered antigens will be present within the scFv libraries. One of the major applications of this approach is to isolate tumor antigen-specific scFvs that may be used for the targeted therapy of canine cancer. Recent reports have revealed that human patients frequently break tolerance to tumor-associated antigens (TAA) and therefore contain TAA-specific Ig in their antigen-experienced repertoire (Felding-Habermann et al., 2004). We therefore have focused our efforts on the generation of antigen-experienced scFv libraries from the spleens of dogs with spontaneous hemangiosarcoma for future attempts to isolate canine scFvs that target antigens specifically expressed on the surface of malignant endothelial cells.

The success of this approach for identifying and isolating scFvs that bind to antigens of interest depends on the ability of the designed primers to amplify a diverse immunoglobulin repertoire. scFv libraries generated in other species have an estimated size of $>1 \times 10^8$ and this size is considered to reflect a large and diverse scFv library (Okamoto et al., 2004; Sepulveda and Shoemaker, 2008; Pansri et al., 2009). Using standard techniques to estimate library size we demonstrate that our primers produce canine scFv libraries of 1×10^8 or greater suggesting that their size is comparable to previous reports of scFv library generation in other species. Furthermore, using BstNI fingerprinting and scFv sequencing we have demonstrated that the generated canine scFv libraries are diverse and contain VH-I and VH-III family members together with members of all the VL lambda gene families (Tables 2 and 3). In accordance with the recent molecular characterization of the canine VH repertoire (Bao et al., 2010), VH sequences belonging to the VH-I family dominated the generated canine scFv repertoire. Furthermore, the generated scFv VH–VLκ libraries contained representatives of the more prominent VLκ gene families. In contrast to humans and mice, the canine immunoglobulin repertoire appears limited in its use of VLκ chain genes, a finding that is based on immunohistochemical analysis of canine lymphoid tissue where the λ:κ Ig ratio was 91:9 (Arun et al., 1996). It is possible in our study that the failure to detect other VLκ gene families within the sequenced VH–VLκ library results from their low frequency of usage and sequencing of an insufficient number of clones to identify rarely used family members. As a result of the predicted low usage of VLκ chains in the canine immunoglobulin repertoire we opted to generate separate VH–VLλ and VH–VLκ libraries to ensure that future efforts to isolate rare, antigen-specific scFvs that utilize VLκ genes will have the best chance of success.

To demonstrate proof-of-principle that the scFv libraries generated using the methods described here contain immunoglobulin binding fragments that target previously encountered antigens, we sought to identify and isolate CPV-specific scFvs from the library of one dog that had previously been vaccinated against CPV. The CPV capsid has been well described and is composed of a combination of VP1 and VP2 proteins (Tsao et al., 1991; Xie and Chapman, 1996). The capsid contains 2 major neutralizing antigenic sites (A and B), and while numerous mouse mAb have been identified and characterized, the sequence and structure of the canine Ab response against CPV is unknown. Two CPV-specific scFv clones were identified in this analysis and we have not yet determined whether these have virus neutralizing capabilities. The finding that the CPV-specific VH sequences represent alleles of the same germline V gene suggests that they may derive from clonally related B cells. In contrast, the VL chains derive from different germline V genes and are therefore unlikely to be clonally related. This suggests that the VH–VL pairing within selected scFv occurs randomly during the second round PCR reaction. Failure to identify additional clones of



(B)

VLλ Sequence

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4
Clone 1	VLTQLPSVSGSLGQRVTISC	IGSSSNVGYGNYVG	WYQQVPGTSPKTLIY	SSDSRPS	GVPDRFSASRSGSTATLTISGLQAEDEADYYC	SSYDTSLSGIV	FGGGTHLTVL
Clone 2	VLTQKASVSGSLGQTITISC	TGTSSNIGHNNVG	WYQQLPGRGPRTVVS	GTNSRPS	GVSDRFSASKSGNTATLTISGLRADDEADYYC	STWDDSLNVV	FGGGTHLTVL

VH Sequence

	FR1	CDR1	FR2	CDR2	FR3	CDR3	FR4	Constant
Clone 1	EGQLAESGGDLVKPGGSLRLSCVASGSTFR	NSHMT	WVRQAPGKGLQWVA	NIDSGAFTTDYVDAVRGRFT	VSRDNARNTMYLQMNSLRAEDTAVYYCAT	MKTSYCIDENCFSFQAGRGVFDK	WGQGTLVTVSS	ASTTAPS
Clone 2	EVQLVESGGDLVKPGGSLRLSCEASGFTFS	NYHMA	WVRQAPGKGLQWVA	NIDSGGFTTNYVDAVRGRFT	VSRDNG--ALYLQMNGLRVEDTAVYYCAT	MKTTYCIDENCNSFQAGRGVFDN	WGQGTLVTVSS	ASTTAPS

Fig. 5. Specificity and amino acid sequences of canine scFvs selected by panning against CPV capsid antigen. (A) Phagemids were rescued from bacterial colonies derived from eluted phage following the fourth round of panning against CPV capsid antigen. CPV-specificity of selected clones was determined by phage ELISA. Phage derived from a pCOMB3X phagemid lacking a scFv and phage expressing a scFv specific for the integrin αVβ3 were used as negative controls (CPV, canine parvovirus; BSA, bovine serum albumin). (B) Open reading frame translations of VLλ and VH immunoglobulin chains within two CPV-specific canine scFvs (the flexible linker has been omitted). Statistical analysis was performed using a two-tailed Student's *T*-test where $^{*}p < 0.0005$.

CPV-specific scFv might indicate that particular Ag-binding regions dominate the Ab response to CPV vaccination. Alternatively, failure to identify a broader Ab response within the scFv library may indicate limitations within the primer sets to identify all framework regions within the canine genome due to (i) gaps within the genome sequence that may contain additional V genes or (ii) due to the stringent search conditions used to identify V genes within the genome.

5. Conclusion

We have developed a series of degenerate primers that aim to amplify the canine antigen-experienced immunoglobulin repertoire. The platform technology described allows for the amplification of VH and VL chains from canine lymphocytes. These are then randomly combined to generate scFvs displayed on the surface of bacteriophage. We have demonstrated that these canine scFv phage display libraries can be screened for specific Ag binders using simple panning techniques and that canine scFvs of interest can be isolated and sequenced. Ag-specific scFvs have the potential for use in a wide range of biomedical applications including the treatment of malignant, infectious, inflammatory and autoimmune diseases (Lerner et al., 1991). The ability to generate canine scFv phage display libraries using the primers described here now paves the way for exploration of such applications in canine medicine.

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References

Arun, S.S., Breuer, W., Hermanns, W., 1996. Immunohistochemical examination of light-chain expression (lambda/kappa ratio) in canine, feline, equine, bovine and porcine plasma cells. ZBL Vet. Med. A 43, 573–576.

Bao, Y., Guo, Y., Xiao, S., Zhao, Z., 2010. Molecular characterization of the VH repertoire in *Canis familiaris*. Vet. Immunol. Immunopathol. 137, 64–75.

Barbas 3rd, C.F., 2001. Phage Display—A Laboratory Manual. Cold Spring Harbor Laboratory Press, New York, USA.

Begent, R.H., Verhaar, M.J., Chester, K.A., Casey, J.L., Green, A.J., Napier, M.P., Hope-Stone, L.D., Cushen, N., Keep, P.A., Johnson, C.J., Hawkins, R.E., Hilson, A.J., Robson, L., 1996. Clinical evidence of efficient tumor targeting based on single-chain Fv antibody selected from a combinatorial library. Nat. Med. 2, 979–984.

Chiang, Y.L., Sheng-Dong, R., Brow, M.A., Larrick, J.W., 1989. Direct cDNA cloning of the rearranged immunoglobulin variable region. Biotechniques 7, 360–366.

Coloma, M.J., Larrick, J.W., Ayala, M., Gavilondo-Cowley, J.V., 1991. Primer design for the cloning of immunoglobulin heavy-chain leader-variable regions from mouse hybridoma cells using the PCR. Biotechniques 11, 152–154, 156.

Davies, J., Riechmann, L., 1996. Single antibody domains as small recognition units: design and in vitro antigen selection of camelized, human VH domains with improved protein stability. Protein Eng. 9, 531–537.

Felding-Habermann, B., Lerner, R.A., Lillo, A., Zhuang, S., Weber, M.R., Arrues, S., Gao, C., Mao, S., Saven, A., Janda, K.D., 2004. Combinatorial antibody libraries from cancer patients yield ligand-mimetic Arg-Gly-Asp-containing immunoglobulins that inhibit breast cancer metastasis. Proc. Natl. Acad. Sci. U.S.A. 101, 17210–17215.

Foord, A.J., Muller, J.D., Yu, M., Wang, L.F., Heine, H.G., 2007. Production and application of recombinant antibodies to foot-and-mouth disease virus non-structural protein 3ABC. J. Immunol. Methods 321, 142–151.

Gao, C., Mao, S., Kaufmann, G., Wirsching, P., Lerner, R.A., Janda, K.D., 2002. A method for the generation of combinatorial antibody libraries using pIX phage display. Proc. Natl. Acad. Sci. U.S.A. 99, 12612–12616.

Haidaris, C.G., Malone, J., Sherrill, L.A., Bliss, J.M., Gaspari, A.A., Insel, R.A., Sullivan, M.A., 2001. Recombinant human antibody single chain variable fragments reactive with *Candida* albicans surface antigens. J. Immunol. Methods 257, 185–202.

Klimka, A., Barth, S., Matthey, B., Roovers, R.C., Lemke, H., Hansen, H., Arends, J.W., Diehl, V., Hoogenboom, H.R., Engert, A., 1999. An anti-CD30 single-chain Fv selected by phage display and fused to *Pseudomonas* exotoxin A (Ki-4(scFv)-ETA′) is a potent immunotoxin against a Hodgkin-derived cell line. Br. J. Cancer 80, 1214–1222.

Knackmuss, S., Krause, S., Engel, K., Reusch, U., Virchow, J.C., Mueller, T., Kraich, M., Little, M., Luttmann, W., Friedrich, K., 2007. Specific inhibition of interleukin-13 activity by a recombinant human single-chain immunoglobulin domain directed against the IL-13 receptor alpha 1 chain. Biol. Chem. 388, 325–330.

Lerner, R.A., Barbas 3rd, C.F., Kang, A.S., Burton, D.R., 1991. On the use of combinatorial antibody libraries to clone the "fossil record" of an individual's immune response. Proc. Natl. Acad. Sci. U.S.A. 88, 9705–9706.

Mao, S., Gao, C., Lo, C.H., Wirsching, P., Wong, C.H., Janda, K.D., 1999. Phage-display library selection of high-affinity human single-chain antibodies to tumor-associated carbohydrate antigens sialyl Lewisx and Lewisx. Proc. Natl. Acad. Sci. U.S.A. 96, 6953–6958.

Marks, J.D., Hoogenboom, H.R., Bonnert, T.P., McCafferty, J., Griffiths, A.D., Winter, G., 1991. By-passing immunization. Human antibodies from V-gene libraries displayed on phage. J. Mol. Biol. 222, 581–597.

Okamoto, T., Mukai, Y., Yoshioka, Y., Shibata, H., Kawamura, M., Yamamoto, Y., Nakagawa, S., Kamada, H., Hayakawa, T., Mayumi, T., Tsutsumi, Y., 2004. Optimal construction of non-immune scFv phage display libraries from mouse bone marrow and spleen established to select specific scFvs efficiently binding to antigen. Biochem. Biophys. Res. Commun. 323, 583–591.

Orlandi, R., Gussow, D.H., Jones, P.T., Winter, G., 1989. Cloning immunoglobulin variable domains for expression by the polymerase chain reaction. Proc. Natl. Acad. Sci. U.S.A. 86, 3833–3837.

Pansri, P., Jaruseranee, N., Rangnoi, K., Kristensen, P., Yamabhai, M., 2009. A compact phage display human scFv library for selection of antibodies to a wide variety of antigens. BMC Biotechnol. 9, 6.

Payne, A.S., Ishii, K., Kacir, S., Lin, C., Li, H., Hanakawa, Y., Tsunoda, K., Amagai, M., Stanley, J.R., Siegel, D.L., 2005. Genetic and functional characterization of human pemphigus vulgaris monoclonal autoantibodies isolated by phage display. J. Clin. Invest. 115, 888–899.

Pelat, T., Hust, M., Laffly, E., Condemine, F., Bottex, C., Vidal, D., Lefranc, M.P., Dubel, S., Thullier, P., 2007. High-affinity, human antibody-like antibody fragment (single-chain variable fragment) neutralizing the lethal factor (LF) of *Bacillus anthracis* by inhibiting protective antigen–LF complex formation. Antimicrob. Agents Chemother. 51, 2758–2764.

Posey, J.A., Khazaeli, M.B., Bookman, M.A., Nowrouzi, A., Grizzle, W.E., Thornton, J., Carey, D.E., Lorenz, J.M., Sing, A.P., Siegall, C.B., LoBuglio, A.F., Saleh, M.N., 2002. A phase I trial of the single-chain immunotoxin SGN-10 (BR96 sFv-PE40) in patients with advanced solid tumors. Clin. Cancer Res. 8, 3092–3099.

Riano-Umbarila, L., Juarez-Gonzalez, V.R., Olamendi-Portugal, T., Ortiz-Leon, M., Possani, L.D., Becerril, B., 2005. A strategy for the generation of specific human antibodies by directed evolution and phage display. An example of a single-chain antibody fragment that neutralizes a major component of scorpion venom. FEBS J. 272, 2591–2601.

Ridder, R., Schmitz, R., Legay, F., Gram, H., 1995. Generation of rabbit monoclonal antibody fragments from a combinatorial phage display library and their production in the yeast *Pichia pastoris*. Biotechnology (NY) 13, 255–260.

Schluter, S.F., Jensen, I., Ramsland, P.A., Marchalonis, J.J., 2005. Recombinant shark natural antibodies to thyroglobulin. J. Mol. Recognit. 18, 404–412.

Sepulveda, J., Shoemaker, C.B., 2008. Design and testing of PCR primers for the construction of scFv libraries representing the immunoglobulin repertoire of rats. J. Immunol. Methods 332, 92–102.

Tang, L., Sampson, C., Dreitz, M.J., McCall, C., 2001. Cloning and characterization of cDNAs encoding four different canine immunoglobulin gamma chains. Vet. Immunol. Immunopathol. 80, 259–270.

Tsao, J., Chapman, M.S., Agbandje, M., Keller, W., Smith, K., Wu, H., Luo, M., Smith, T.J., Rossmann, M.G., Compans, R.W., et al., 1991. The three-dimensional structure of canine parvovirus and its functional implications. Science 251, 1456–1464.

Wang, Z., Raifu, M., Howard, M., Smith, L., Hansen, D., Goldsby, R., Ratner, D., 2000. Universal PCR amplification of mouse immunoglobulin gene variable regions: the design of degenerate primers and an assessment of the effect of DNA polymerase 3′ to 5′ exonuclease activity. J. Immunol. Methods 233, 167–177.

Xie, Q., Chapman, M.S., 1996. Canine parvovirus capsid structure, analyzed at 2.9 Å resolution. J. Mol. Biol. 264, 497–520.

Genetic Tests: How to Interpret Results and Incorporate Them Into Your Breeding Program

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Genetic tests vary on what they are able to identify, and therefore how they can be used in managing genetic disease.

Phenotypic tests: Some tests measure the phenotype, or what can be seen in the animal. This may not directly relate to the genotype, or the genes regulating the defect that you are trying to manage. Screening for cataracts, ausculting for heart murmurs, hip and elbow radiographs, thyroid profiles, urinalysis for crystals or metabolites, skin biopsy for sebaceous adenitis, and observations on behavioral traits are all tests of the phenotype. Most tests of the phenotype only identify affected individuals, and not carriers of disease liability genes.

Linked-marker based tests: Some defective genes can be linked to a genetic marker, which could be tested for. Linked-marker based tests do not identify the defective gene, but a marker that lies close on the chromosome. If a crossover occurs between the marker and the defective gene during reproduction, the marker will no longer be linked to the defective gene. False positive and false negative results will occur. Due to this phenomenon, linkage test results must be compared with results from other family members to determine whether they correlate with the known genotype of relatives. Linked marker tests include those for cerebellar ataxia in Italian Spinone and primary hyperparathyroidism in Keeshond.

Direct mutation based tests: Direct gene tests are specific for mutations and are a direct measurement of the genotype. They can identify affected, carrier, and normal individuals. These can be run at any age, regardless of the age of onset of the disorder.

Most direct gene tests identify a mutation that is causative for a genetic disorder. However, some direct genetic tests identify a mutation that causes an increased susceptibility for genetic disease. These **susceptibility alleles** can be part of polygenic/complexly inherited traits, or the cause of incomplete penetrance of (assumed) simple Mendelian traits.

Examples are cord1 PRA in English Springer Spaniels and Miniature Dachshunds, and degenerative myelopathy in several dog breeds. Degenerative myelopathy is considered a complexly inherited disease. An autosomal recessive susceptibility gene has been identified that is homozygous (two abnormal copies) in all DM affected dogs. However, a large proportion of individuals in these breeds are homozygous for this gene and do not become affected. They are considered “at risk”, but not genetically affected. In the Boxer, less than 0.5% of dogs develop the disease. Testing Boxers for the DM susceptibility gene shows 39% testing carrier, and 43% testing homozygous for the susceptibility gene. This test is useful in determining individual dogs with and without risk of developing DM. However, selecting against 82% of the Boxer gene pool when making breeding decisions, when the majority will not produce the disorder is detrimental to the genetic diversity of the breed.

Other susceptibility genes are found to occur at a greater frequency in affected animals, but **are not present in all affected animals.** An example is the susceptibility gene for perianal fistula/anal furunculosis in German Shepherd Dogs. Dogs with the susceptibility haplotype (specific sequence of genes) have a 3.7X odds ratio for the disease versus those without the haplotype. Another example is the genetic test for Pug Dog Encephalitis, where dog homozygous for a susceptibility haplotype have a 12.7X odds ratio for developing the disease.

Utilizing Genetic Tests

We need to be knowledgeable about what genetic tests are available, and in which individuals they should be run. Dogs from breeds with an incidence of von Willebrand disease should be tested early in life, so that measures can be taken to prevent excessive hemorrhage during surgery or injury. Dogs in breeds at risk of carrying the mdr-1 drug sensitivity mutation should be tested early in life, before drug treatment. In high-risk breeds for serious genetic disease, individual animals should be genetically tested (or verified results documented for parents) before purchase. These include Boxers for arrythmogenic right ventricular cardiomyopathy, and Doberman Pinschers for dilated cardiomyopathy.

We need to understand the temporal periods when genetic testing will be most accurate, and allow for intervention. Genetic testing for inherited hypothyroidism (autoimmune thyroiditis) is based on the presence of thyroglobulin autoantibodies. A dog with normal TgAA levels on two tests at least two years apart between two and six years of age is phenotypically normal. However, TgAA levels should not be measured within 2-3 months post-vaccination, as a transient iatrogenic rise can occur during this period.

For most genetic diseases, we know how to either prevent their occurrence, or at least lessen the possibility of producing offspring with genetic disease. This can occur through the genotypic testing of the parents (identification of parents carrying liability genes for genetic disease), phenotypic testing of the parents (identification of parents affected with genetic disease), or pedigree analysis (identification of carrier and affected risk based on the knowledge of carrier or affected relatives).

The genetic improvement of dogs will only occur through selective breeding. Inherent in this point is the acknowledgement that breeding without genetic testing is irresponsible, and unethical. **Genetic testing is health quality control.** It is no longer acceptable for a breeder to choose two individuals and breed them together without regard to genetic disease control. The most important goal of managing genetic disease is to avoid producing affected individuals. The secondary goal is to reduce the frequency of carriers of defective genes in the population. At the same time, recommendations should allow perpetuation of breeding lines, in order to preserve the genetic diversity of the population.

With each new generation, breeders ask, “How can I continue my line and improve it?” Aside from selecting for conformation, behavior and general health, breeders must consider how they are going to reduce the incidence of whichever genetic disorders are present in their breed. There are no answers that will fit every situation. There are, however, guidelines to preserve breeding lines and genetic diversity while reducing the risk of producing animals that carry defective genes, or are affected with genetic disorders.

Autosomal Recessive Disorders

In the case of a simple autosomal recessive disorder for which a *direct genetic test for carriers* is available, the recommendation is to test breeding-quality stock, and breed normals to normals, or carriers to normal-testing individuals. This prevents affected offspring from being produced.

Breeders are the custodians of their breed's past and future. "Above all, do no harm" is a primary oath of all medical professionals. Genetic tests are powerful tools, and their use can cause significant positive or negative changes to breed gene pools. Once a genetic test is developed that allows breeders to determine if an animal is a carrier of a defective gene, many owners are likely to simply eliminate carriers from breeding. Although doing so is human nature, this temptation must be overcome. If an owner would breed an individual if it tested normal for a genetic disease, then a carrier result should not change that decision. A direct genetic test should not alter WHO gets bred, only WHO THEY GET BRED TO. One defective gene that can be identified through a genetic test out of tens of thousands of genes is not a reason to stop breeding. A genetic test that should be used to help maintain breed quality and diversity should not result in limiting it.

We know that most individuals carry some unfavorable recessive genes. The more genetic tests that are developed, the greater chance there is of identifying an undesirable gene in a breeding animal. History has shown that breeders can be successful in reducing breed-wide genetic disease through testing and making informed breeding choices. However, there are also examples of breeds that have actually experienced more problems as a result of unwarranted culling and restriction of their gene pools. These problems include: 1) Reducing the incidence of one disease and increasing the incidence of another by repeated use of males known to be clear of the gene that causes the first condition. 2) Creating bottlenecks and diminishing diversity by eliminating all carriers of a gene from the breeding pool, instead of breeding and replacing them. 3) Concentrating on the presence or absence of a single gene and not the quality of the whole animal.

The aim is to replace the carrier breeding-animal with a normal-testing offspring that equals or exceeds it in quality. Additional carrier testing offspring should not be placed in breeding homes; as the goal is to reduce the frequency of the defective gene in the population. As each breeder tests and replaces carrier animals with normal-testing offspring, the problem for the breed as a whole diminishes, while not restricting gene pool diversity.

The problem with a simple autosomal recessive disorder for which *no carrier test exists* is the propagation and dissemination of unapparent carriers in the gene pool. A quality individual that is found to be a carrier of a recessive gene can be retired from breeding and replaced with a quality relative or prior-born offspring. The genes of the retired individual can thus be preserved through the selected relative, but the carrier risk can be cut in half. To further limit the spread of the defective gene, the offspring should be used in only a limited number of carefully planned matings, and then should also be replaced with one or two representative offspring. The rest of the litter should be placed in non-breeding (pet) homes. With this mating scheme, you are maintaining the good genes of the line, reducing the carrier risk with each generation, and replacing, not adding to the overall carrier risk in the breeding population.

Breeders must assess the carrier risk of each individual animal in their breeding program. An open health registry that is supported by the parent club makes it easier for breeders to objectively assess these matters. An example is the genetic

disease control program for cerebellar abiotrophy by the Scottish Terrier Club of America (http://www.stca.biz/health-registries/ca-registry). By determining the average carrier-risk for the breeding population, breeders can select matings that have a projected risk that is lower than the breed average. Relative risk assessments only take into account the identified carrier and affected individuals in the pedigree. Therefore, these estimates determine the minimum risk based on the information available. If additional affected relatives to the pedigree are diagnosed, the computed risk will rise. The relative risk pedigree calculator on the Scottish Terrier website can be used by any breed to compute carrier and affected risk for any simple autosomal recessive disorder.

If a quality breeding animal is at high risk of being a carrier, the best advice is to breed to an individual that has a low risk. Using relative-risk assessment as a tool, breeders should replace higher-risk breeding animals with lower-risk offspring that are equal to or better than their parents in quality. A negative aspect of pedigree analysis is that it selects against families, regardless of an individual's normal or carrier status. On the other hand, it allows for the objective risk assessment and continuation of lines that might otherwise be abandoned due to high carrier-risk.

Autosomal Dominant Disorders

Autosomal dominant genetic disorders are usually easy to manage. Each affected animal has at least one affected parent, but it can be expected that half of the offspring of an affected animal will be free of the defective gene. With disorders that cause death or discomfort, the recommendation is to not breed affected animals. To produce the next generation of a line; a normal full sibling to an affected animal, a normal close relative, or the parent that is normal can be used.

If the defective gene is at a high frequency in the gene pool, eliminating all affected breeding animals in one generation may have a significant negative impact on genetic diversity. When a high frequency autosomal dominant disorder is first identified, some quality, affected animals may have to be bred, and replaced with quality, normal testing offspring. However, once a few generations have gone by and breeders have had the opportunity to replace affected with normal individuals, the continued breeding of affected animals is not ethical.

A problem with some autosomal dominant disorders is incomplete penetrance; where some animals with the defective gene may not show the disorder. Roughly half their offspring, however, may be affected. If a genetic test is available, this is not a problem. Otherwise, pedigree analysis and relative-risk assessment can identify which animals are at risk of carrying incompletely penetrant dominant genes.

Sex-Linked Disorders

For sex-linked (also known as x-linked) recessive defective genes for which carrier tests exist, breeders should follow the same "breed and replace" recommendations as are outlined above in the discussion of autosomal recessive disorders. If there is no test, the defective gene can be traced through the pedigree. Selecting a normal male for breeding loses the defective gene in one generation, regardless of his relationship to affected and carrier relatives. Carrier, affected, or high risk females should not be used, due to the high risk of producing affected male offspring. If a male is affected, he would have received the defective gene from his carrier mother. All of his daughters will be carriers, but none of his sons. Without a test for carriers, you can use relative-risk assessment to breed him to a female that is at low risk of being a carrier. This minimizes the chance of producing affected offspring, and a quality son can be selected for replacement. Rare sex-linked dominant disorders are managed the same way as autosomal dominant disorders. The difference is that affected males will always produce all affected daughters.

Polygenic disorders/Complex Inheritance

Polygenic disorders are those caused by more than one pair of genes. A number of liability genes must combine to cross a threshold and produce an affected individual. Most polygenic disorders have no tests for carriers, but they do have phenotypic tests that can identify affected individuals. Controlling polygenically inherited disorders involves; 1) identifying traits that more closely represent genes being selected against, 2) the standardization of nuisance factors (such as environment) that can limit your selective pressure against the genes and 3) selecting for breadth of pedigree as well as depth of pedigree.

In polygenic disorders, the phenotype of the individual does not directly represent its genotype. If phenotypically normal parents produce affected offspring, both should be considered to carry a genetic load of liability genes that combined to cause the disorder. Breeders must break down affected phenotypes into traits that more directly represent the genes that control them. For example, in hip dysplasia these can include clinical signs of lameness, shallow hip sockets, subluxation or remodeling on an extended leg view, and radiographic distractibility on a PennHIP view. If a quality individual is to be bred, but has shallow

hip sockets, it should be bred to an individual with deep hip sockets. You need to select for enough genes influencing normal development, to get below the threshold where dysplasia develops.

The environment has a role in the expression of polygenic disorders. Plane of nutrition and environmental stress, especially during critical growth periods can alter the expression of some inherited musculoskeletal disorders. You do not want to overly protect or overly stress the development of prospective breeding animals. Breeders should evaluate prospective breeding individuals raised under fairly uniform conditions, which will not mask or alter the expression of genetic disease.

Polygenic disorders require knowledge of the affected or normal status of full-siblings to prospective breeding animals. Individuals whose siblings are normal and whose parents' sibs are normal have the greatest chance of carrying a low genetic load for the condition. This *breadth of pedigree* analysis is more important than normalcy in the depth of pedigree (parents and grandparents only.) This is why it is important to screen both pet and breeding animals from litters for polygenic disorders, and report the results in open health registries, such as the not-for profit Orthopedic Foundation for Animals (www.offa.org). Breadth of pedigree results can be visualized by clicking on vertical pedigrees on the individual dog's OFA page.

Affected individuals can be replaced with a normal sib or parent, and bred to a low-liability mate. Breeders can replace the higher risk parent with a quality, lower risk offspring, and repeat the process. In addition, the offspring of breeding dogs should be monitored to see which are passing the disorder with higher frequency.

Undetermined Mode of Inheritance

For disorders without a known mode of inheritance or carrier test, breeders should be counseled to use the same control methods as with polygenic disorders. Animals with a low genetic load for the disorder should be selected for breeding, through the results of examinations of first-degree relatives (littermates, parents, and offspring). If there are multiple generations of normalcy in the breadth of the pedigree, then you can have some confidence that there is less risk that liability genes are being carried.

It is distressing when a genetic disorder is confirmed. Positive and practical genetic counseling recommendations can be made to maintain breed lines and genetic diversity, and improve the overall health of breeds. The total elimination of defective genes will probably be impossible for most breeds. The use of these guidelines can assist breeders in making objective breeding decisions for genetic disease management, while continuing their breeding lines. The individual breeder can use genetic tests to; 1) identify carriers, 2) work to breed away from the defective gene(s), and 3) ensure (through testing) that the defective gene(s) is not reintroduced in future matings. Each breeder will have their own rate of progress, depending on the frequency of the defective gene(s) in their own breeding animals, and which desirable individuals carry liability genes.

Small Population Breeds and Issues of Genetic Diversity

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Issues of genetic diversity are a concern to dog breeders, and this can especially be so for breeds with small populations. The concern is whether there is enough genetic variation within a breed's gene pool to maintain health and vitality. Breeders should be concerned about genetic diversity, because there are examples where damage has been done to a breed due to breeding practices. Restriction of genetic diversity can also occur in large population breeds.

All genes come in pairs: one from the sire and one from the dam. Each gene in the pair is called an allele. If both alleles in a pair are of the same type, the gene pair is homozygous. If the two alleles are different, the gene pair is heterozygous. While each dog can have a maximum of two different alleles at a gene pair, many different alleles are potentially available to be part of the gene pair. The greater the number of alleles that are available at each gene pair (called genetic polymorphism), the greater the genetic diversity of the breed.

If there is no breed diversity in a gene pair, but the particular homozygous gene that is present is not detrimental, there is no negative effect on breed health. The characteristics that make a breed reproduce true to its standard are, in fact, based on nonvariable (that is, homozygous) gene pairs.

The origins of breeds have a lot to do with genetic diversity. A breed established with a working phenotype tends to have diverse founder origins, and significant diversity. Even with substantial population bottlenecks, the breed can maintain considerable amounts of genetic diversity. This was shown in a molecular genetic study of the Chinook breed, which was reduced to 11 modern founders in 1981. Breeds established by inbreeding on a limited number of related founder individuals could have reduced diversity. Many breeds have also gone through diversity reducing bottlenecks; such as occurred during World War II. For most of these breeds, their gene pools have expanded through breeding for many generations, resulting in a stable population of healthy dogs.

There are two factors that must be considered when evaluating genetic diversity and health issues in a breed; the average level of inbreeding, and detrimental recessive genes. With a small population, there is a tendency to find higher average inbreeding coefficients due to the relatedness between dogs from common ancestors. There is, however, no specific level or percentage of inbreeding that causes impaired health or vigor. The problems that inbreeding depression cause in purebred populations stem from the effects of deleterious recessive genes. If the founding population of a breed produces a high frequency of a deleterious recessive gene, then the breed will have issues with that disorder. This can be seen as smaller litter size, increased neonatal death, high frequency genetic disease, or impaired immunity. If these issues are present then the breed needs to seriously consider limited genetic diversity.

The issue of high average inbreeding coefficients is one that all breeds go through during their foundation. As the population increases and the average relatedness of dogs goes down (based on a fixed number of generations), the average inbreeding coefficient for the breed will go down. The effect of initially higher inbreeding coefficients in small population breeds will depend on the presence of deleterious recessive genes that will be expressed when homozygous.

Some breeders discourage linebreeding and promote outbreeding in an attempt to protect genetic diversity in their breed. It is not the type of matings utilized (linebreeding or outbreeding) that causes the loss of genes from a breed gene pool. Rather, loss of genes occurs through selection: the use and non-use of offspring. If a breed starts narrowing their focus to breeding stock from a limited number of lines, then a loss of genetic diversity will occur.

The process of maintaining healthy lines, with many breeders crossing between lines and breeding back as they see fit, maintains diversity in the gene pool. If some breeders outbreed, and some linebreed to certain dogs that they favor while others linebreed to other dogs that they favor, then breedwide genetic diversity is maintained. It is the varied opinion of breeders as to what constitutes the ideal dog, and their selection of breeding stock based on their opinions, that maintains breed diversity.

The most important factor for diminished genetic diversity in dog breeds is the popular sire syndrome. The overuse of a popular sire beyond a reasonable contribution through frequent breedings significantly skews the gene pool in his direction, and reduces the diversity of the gene pool. Any genes that he possesses - whether positive or negative - will increase in frequency. Through this founder's effect, breed-related genetic disease can occur. Another insidious effect of the popular sire syndrome is the loss of genetic contribution from quality, unrelated males who are not used for breeding. There is a finite number of quality bitches bred each year. If one male is used in an inordinate amount of matings, there will be fewer females left for these quality males that should be contributing to the gene pool. The popular sire syndrome is a significant factor in both populous breeds and breeds with small populations.

The best methods for ensuring the health and diversity of any breed's gene pool are to: 1) Avoid the popular sire syndrome. 2) Utilize quality dogs from the breadth of your population to expand the gene pool. 3) Monitor genetic health issues through regular health surveys. 4) Do genetic testing for breed-related disorders. 5) Participate in open health registries, such as CHIC (www.caninehealthinfo.org) to manage genetic disorders.

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How the Orthopedic Foundation for Animals (OFA) is tackling inherited disorders in the USA: Using hip and elbow dysplasia as examples

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ABSTRACT

The Orthopedic Foundation for Animals (OFA) maintains an on-line health pedigree database for inherited disorders of animals. With the American Kennel Club Canine Health Foundation, the OFA maintains the Canine Health Information Center (CHIC) for parent breed clubs to identify breed-specific required health tests. Analysis of the results of OFA evaluations in the hip and elbow registries show that selection based on phenotype improves conformation. Disorders with complex inheritance respond best to selection based on depth (ancestors) and breadth (siblings) of pedigree health test results. This information can be derived from vertical pedigrees generated on the OFA website.

Introduction

A prominent businessman in the United States, John M. Olin, was also an avid sportsman and recognized the impact of canine hip dysplasia on his Labrador retrievers. Along with the Golden Retriever Club of America, German Shepherd Club of America and the veterinary community, he organized a meeting that eventually led to the formation of the Orthopedic Foundation for Animals (OFA) in 1966. The OFA is guided by the following four specific objectives:

(1) To collate and disseminate information concerning orthopedic and genetic diseases of animals.
(2) To advise, encourage and establish control programs to lower the incidence of orthopedic and genetic diseases.
(3) To encourage and finance research in orthopedic and genetic disease in animals.
(4) To receive funds and make grants to carry out these objectives.

The OFA is governed by a voluntary Board of Directors. As a not-for-profit organization, the revenue over expenses is either held in the operating reserve or donated to support animal health-related research. Most funding is channeled through the American Kennel Club Canine Health Foundation (AKC-CHF)[1] or Morris Animal Foundation, with occasional direct funding. OFA has supported research not only in orthopedic diseases but also for cancer, cardiac, hepatic, nephritic, neurologic, ocular and thyroid disease.

While the OFA's initial focus was canine hip dysplasia, the mission has broadened to include cats and other genetic diseases, including elbow dysplasia, patella luxation, autoimmune thyroiditis, congenital heart disease, Legg–Calve–Perthes disease, osteochondrosis dissecans (shoulder osteochondrosis), sebaceous adenitis and congenital deafness. The methodology and criteria for evaluating the test results for each disorder are independently established by veterinary scientists from their respective specialty areas and the standards used are generally accepted throughout the world. Disorders present on the OFA website include those that have a defined test for normalcy. Disorders such as epilepsy, gastric dilatation/volvulus and cancers that do not have defined phenotypic or genotypic tests are not included. If genetic markers for disease liability are identified in the future, these can be added as tools for genetic disease control.

The power of the OFA genetic database lies in the compilation and integration of all health screening information in a single location. For dogs with an existing OFA record, examination results from the Canine Eye Registry Foundation (CERF) are incorporated in their OFA record. In addition, the results of genotypic tests that are either submitted by the owner or through a cooperative agreement with the parent club are also included in the OFA genetic database. Cutting-edge advancements in molecular genetics now account for over 90 DNA tests involving over 145 breeds of dogs and cats.

The collection of such data is meaningless unless the data can be disseminated to parties of interest. The OFA maintains an

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[1] See: www.akcchf.org.

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on-line database of >1 million phenotypic and genotypic test results.[2] All normal or grades of normal results in the OFA database are available on-line. Abnormal or grades of abnormal results are available on-line if released by the owner, or if the results are part of a breed club program where all (normal and abnormal) test results are published.

The Canine Health Information Center (CHIC)[3] is a program that is dually sponsored by the OFA and the AKC-CHF. The parent clubs determine the breed-specific health issues for CHIC certification and encourage breeder participation in the program. The CHIC program is not about normalcy; it is about health consciousness. Dogs receive CHIC certification if they have completed the required breed-specific health testing, regardless of the test results. Other requirements include permanent identification (tattoo or microchip) and release to the open database of abnormal results. CHIC encourages health screening to improve the overall health of breeds. There are presently over 139 parent breed clubs participating, with over 64,500 dogs achieving CHIC certification.

The acceptance of the CHIC certification program by parent breed clubs and breeders provides an avenue for the only proven method of genetic disease control: breed-specific phenotypic and genotypic screening of prospective breeding stock. The CHIC program provides a standard for breeders to practice health-conscious breeding. It also allows pet owners to screen prospective purchases for evidence of health-conscious breeding.

Another goal of the CHIC program is to collect and store canine DNA samples, along with corresponding genealogic and phenotypic information, to facilitate future research and testing aimed at reducing the incidence of inherited disease in dogs. Researchers have been hampered by the lack of appropriate DNA samples and the DNA repository addresses this need. To date, the CHIC DNA Repository contains DNA from over 12,500 dogs and has received 17 requests from researchers, resulting in the distribution of over 2,200 DNA samples with their appropriate health and pedigree information.

To evaluate hip dysplasia, the OFA employs the ventrodorsal hip-extended positioning recommended by the American Veterinary Medical Association (AVMA Council on Veterinary Service, 1961). The in-house radiologist is the sole evaluator for preliminary evaluation of dogs <24 months of age. The reliability of preliminary hip evaluations for predicting of-age OFA ratings was demonstrated by Corley et al. (1997). Dogs or cats must be $\geqslant$24 months of age to receive OFA hip certification. Radiographs are independently evaluated by three board-certified veterinary radiologists out of a pool of consultants maintained by the OFA. The consensus rating of these three radiologists becomes the hip rating that is reported to the owner and referring veterinarian. There is a high degree of inter- and intra-reader correlation for conventional and digital images (Corley, 1992; Essman and Sherman, 2006).

Seven OFA hip ratings are reported: Excellent, Good, Fair, Borderline, Mild, Moderate or Severe. The first three ratings are considered to be normal, while the last three ratings are regarded as dysplastic. A Borderline rating is given when there is no clear consensus between radiologists to place the hips in a category of normal or dysplastic. It is recommended that dogs with this rating have a repeat radiograph submitted after a minimum of 6 months.

The OFA elbow dysplasia registry employs the protocol established by the International Elbow Working Group (IEWG),[4] which consists of Normal or Grades I, II or III Dysplastic based on the severity of secondary osteoarthritis/degenerative joint disease present on an extreme flexed mediolateral view (International Elbow Working Group, 2001). When a specific component of elbow dysplasia is observed, it is reported in addition to the Grade as ununited anconeal process, osteochondrosis or fragmented medical coronoid process. Elbow radiographs are subjected to the same of-age or preliminary evaluation and certification process as hip radiographs.

Diseases with complex inheritance can respond to selective pressure based on phenotype (Keller, 2006; Pirchner, 1983). In this manuscript, the OFA hip and elbow registries are used to illustrate this response.

Materials and methods

The OFA hip registry of 1,187,831 evaluations was queried for hip ratings of progeny where both parents also had known of-age hip ratings. Data were collected on progeny with of-age or preliminary hip confirmation ratings of normal (Excellent, 1; Good, 2; Fair, 3) or dysplastic (Mild, 5; Moderate, 6; Severe, 7). Progeny with Borderline (4) hip ratings were not included. The hip ratings of both parents were recorded, including all seven grades. A hip Combined Parent Score (CPS) for each mating was determined by adding together the numbers corresponding to the hip rating for each parent; for two OFA Excellent parents the CPS was 2 and for two OFA Severe parents the CPS was 14. Matings with the same CPS were combined together for analysis; e.g. Good mated to Borderline, Fair mated to Fair and Excellent mated to Mild all have a CPS of 6.

The OFA elbow registry of 260,195 evaluations was queried for elbow ratings of progeny where both parents had known of-age elbow ratings. Data were collected on progeny with preliminary or of-age elbow confirmation ratings of Normal (1) or dysplastic (Grade I, 2; Grade II, 3; Grade III, 4). An elbow CPS for each mating was determined by adding together the numbers corresponding to the elbow rating for each parent; for two OFA Normal parents the CPS was 2 and for two OFA Grade III parents the CPS was 8. Matings with the same CPS were combined together for analysis.

Pearson correlation analysis was performed to compare the CPS of matings to the observed percentages of hip dysplasia or elbow dysplasia in the progeny.

Results

Table 1 shows the hip ratings for 490,966 progeny in the OFA hip registry with known sire and dam hip ratings. The percentage of dysplastic progeny increased as the parental hip scores increased. The total number of hip radiograph submissions from parents with normal hip ratings was significantly greater than those from parents with dysplastic hip ratings ($P > 0.05$).

Fig. 1 shows the relationship between the CPS and the percentage of dysplastic progeny. Matings with the same CPS (on the diagonal of Table 1) were strongly correlated with increasing percentages of dysplastic progeny (Pearson correlation coefficient $r = 0.96$; $P > 0.05$). The single CPS that did not reflect this trend was for matings between two severely dysplastic parents, where only 18 progeny were submitted for evaluation.

Table 2 shows the elbow ratings for 67,599 progeny in the OFA elbow registry with known sire and dam elbow ratings. Matings including one normal parent had significantly lower percentages of progeny with elbow dysplasia (12.4%) than those between two parents with elbow dysplasia (45.4%) ($P > 0.05$). Matings involving a parent with Grade I elbow dysplasia produced significantly more elbow dysplasia (25.6%) than matings including a parent with normal elbows ($\chi^2 = 0.77$, 6 df, $P = 0.99$).

Fig. 2 shows the relationship between the CPS and the percentage of progeny with elbow dysplasia. The Pearson correlation coefficient between the CPS and percentage of dysplastic progeny was $r = 0.06$. The lack of correlation is due to the low percentage of dysplasia in progeny of Grade III sires bred to Grade II dams, and Grade III parents bred to each other. The total number of progeny from these matings numbered 14 and 3, respectively.

Discussion

The OFA hip data and CPS demonstrate that hip dysplasia is inherited in an additive and quantitative manner. This verifies

[2] See: www.offa.org.
[3] See: www.caninehealthinfo.org.
[4] See: www.iewg-vet.org/.

Table 1
Progeny results of matings between parents with known hip scores.

Sire rating	Dam rating							Total
	Excellent (1)	Good (2)	Fair (3)	Borderline (4)	Mild (5)	Moderate (6)	Severe (7)	
Excellent (1)								
Dysplastic (%)	3.6	6.1	9.6	12.3	13.4	18.7	18.5	
Total	17,972	52,784	9039	155	1271	729	65	82,015
Good (2)								
Dysplastic (%)	5.8	9.6	14.6	17.5	18.9	23.0	31.5	
Total	50,485	217,938	49,212	811	6930	3973	461	329,810
Fair (3)								
Dysplastic (%)	9.4	14.1	19.8	22.8	26.5	32.2	37.1	
Total	6241	41,628	13,513	263	2301	1328	167	65,441
Borderline (4)								
Dysplastic (%)	8.9	17.7	20.2	22.2	30.8	50.0	50.0	
Total	79	532	168	9	39	30	4	861
Mild (5)								
Dysplastic (%)	16.4	18.3	27.2	36.2	29.6	41.4	45.0	
Total	807	4531	1532	47	459	239	40	7655
Moderate (6)								
Dysplastic (%)	18.9	22.8	31.6	34.4	35.0	38.0	65.3	
Total	428	2618	896	32	266	213	49	4502
Severe (7)								
Dysplastic (%)	22.0	24.2	36.0	44.4	39.6	55.8	44.4	
Total	59	360	136	9	48	52	18	682
Total	76,071	320,391	74,496	1326	11,314	6564	804	490,966

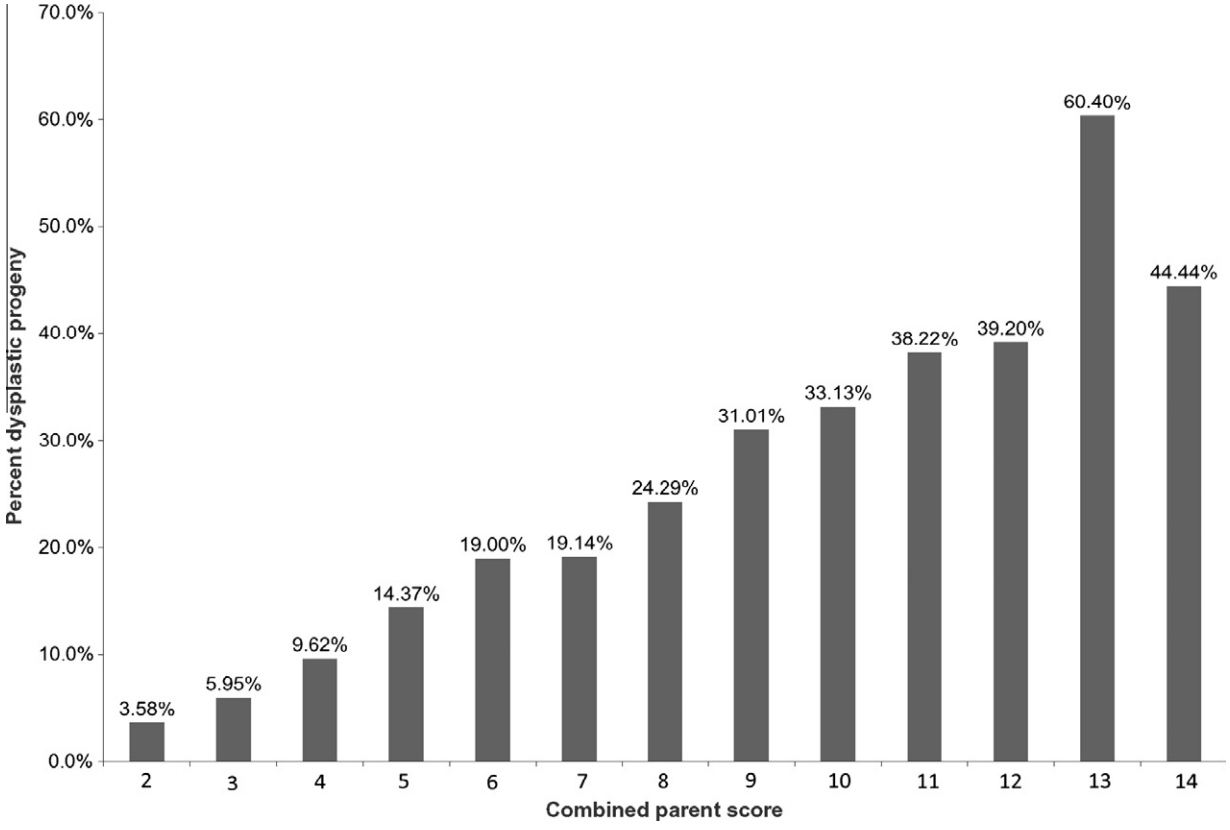

Fig. 1. Relationship of Combined Parent Score to percentage of hip dysplastic progeny.

the conclusions of other researchers that canine hip dysplasia is inherited as a quantitative trait (Leighton, 1997; Zhu et al., 2009; Hou et al., 2010). Hou et al. (2010) analyzed all Labrador retrievers in the open-access OFA hip database and calculated an heritability of 0.21, which confirms hip dysplasia acting as a moderately heritable disease. They also confirmed a steady genetic improvement of OFA hip ratings in the breed over a 40 year period. These results validate the OFA recommendation that using parents with better phenotypic hip conformation produces offspring with better hips.

It was expected that fewer radiographs would be submitted for the progeny of two dysplastic parents, since fewer breeders perform such matings. The low numbers may also be due to pre-

Table 2
Progeny results of matings between parents with known elbow scores.

Sire rating	Dam rating				Total
	Normal (1)	Grade I (2)	Grade II (3)	Grade III (4)	
Normal (1)					
Dysplastic (%)	10.1	24.1	29.4	28.1	
Total	55,867	4309	875	167	61,218
Grade I (2)					
Dysplastic (%)	22.0	41.0	46.9	52.2	
Total	3917	591	145	23	4676
Grade II (3)					
Dysplastic (%)	32.6	55.4	65.8	57.1	
Total	1121	222	38	14	1395
Grade III (4)					
Dysplastic (%)	23.9	38.1	14.3	0.0	
Total	251	42	14	3	310
Total	61,156	5164	1072	207	67,599

screening of radiographs with obviously dysplastic hips by veterinarians; these radiographs may not be submitted to the OFA for evaluation (Paster et al., 2005). This would reduce the resultant frequencies of dysplastic individuals. Prescreening of dysplastic radiographs for OFA submission appears to be constant over time (Reed et al., 2000).

Traits such as hip dysplasia and elbow dysplasia are complexly (polygenically) inherited, with increasing incidence based on increasing frequencies of susceptibility alleles at loci that contribute to variation in liability. Selection based on vertical or depth-of-pedigree hip ratings (parents and grandparents), when combined with an individual's own rating, increases the accuracy of selection and hence response to selection. Similarly, selection based on horizontal or breadth-of-pedigree hip ratings (siblings), when combined with an individual's own rating, increases accuracy of selection and hence response to selection (Pirchner, 1983; Keller, 2006).

Breeding schemes that employ estimated breeding values (EBVs) that combine phenotypic ratings from all known relatives (weighted according to genetic relationship) provide the greatest selective power, rather than single measurements on individual dogs (Zhu et al., 2009; Hou et al., 2010). EBVs that utilize molecular genetic markers for liability genes would be even more beneficial (Stock and Distl, 2010; Zhou et al., 2010).

The open-access OFA health database website provides breeders with the information that helps them to make informed breeding decisions. When an individual dog's record is accessed, detailed information on all recorded health issues, including test results, age at the time of testing and the resulting certification numbers, are available. Sire and dam information are provided, as well as information on full and half siblings and any offspring that may be in the database. A vertical pedigree can be generated from a link on the individual's OFA page, providing traditional depth of pedigree and breadth of pedigree health information. This type of data is extremely useful when trying to make selection decisions based on phenotypic data.

The vertical hip pedigree of the Golden retriever Champion (Ch.) Faera's Starlight (Fig. 3) shows how parent, grandparent, offspring and sibling information are combined in a single graphic format for evaluation. Whilst this dog had hips with an Excellent rating, he was bred from Fair- and Good-rated parents, with three Fair- and one Good-rated grandparents. While he produced 92.4% normal offspring with a preponderance of Good ratings, he produced more Fair- than Excellent-rated offspring. The vertical pedigree provides more information than the single individual rating. Vertical

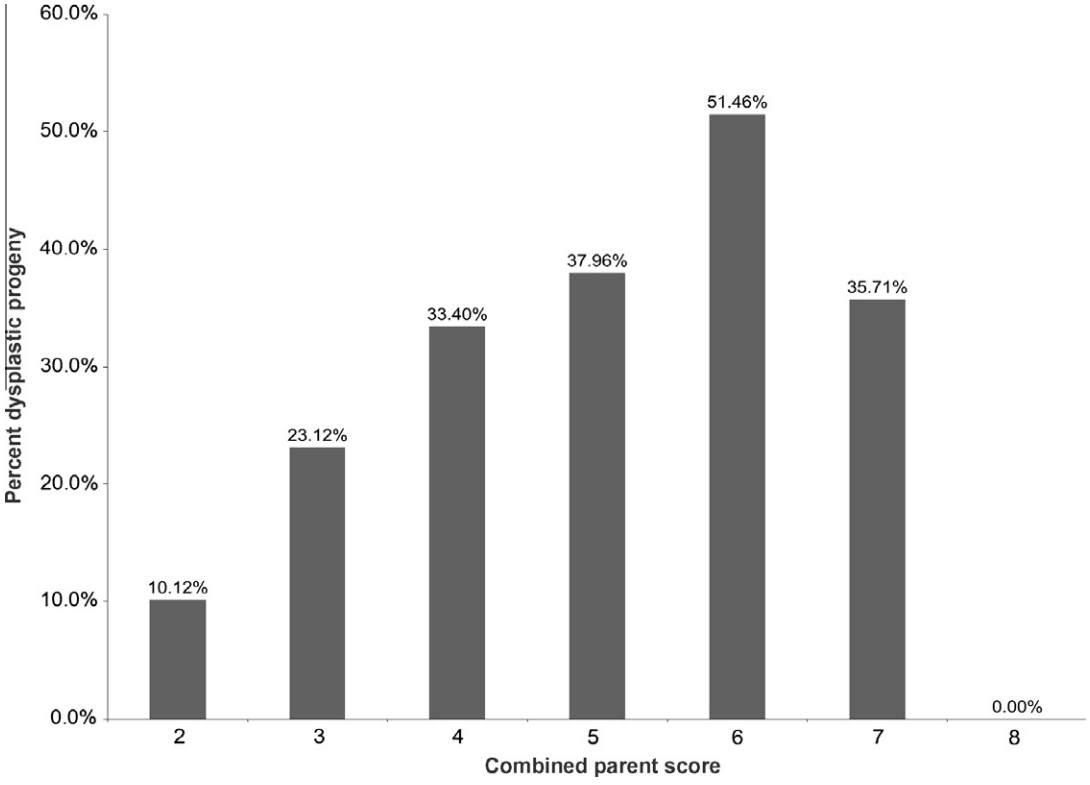

Fig. 2. Relationship of Combined Parent Score to percentage of elbow dysplastic progeny.

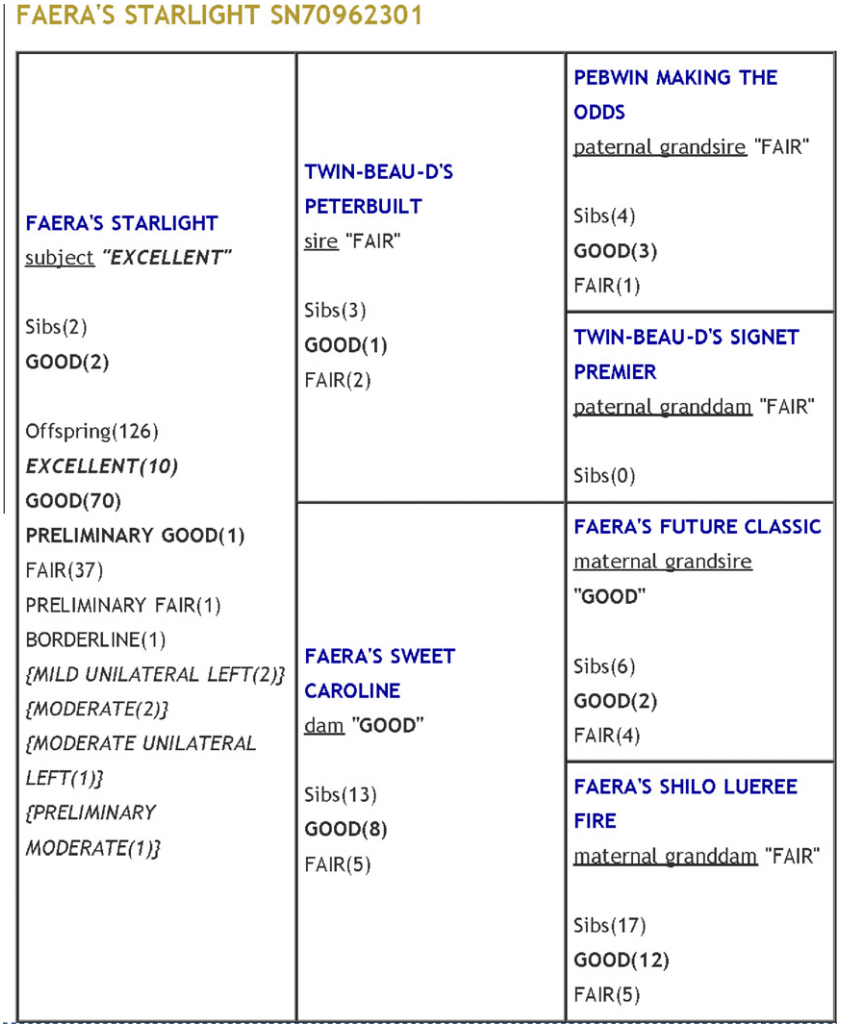

Fig. 3. OFA vertical pedigree of Golden retriever Ch. Faera's Starlight.

pedigrees of individual animals are available on the OFA website for the hip, elbow, cardiac, thyroid, patella, CERF (eye) and degenerative myelopathy registries.

EBV technology would combine all of the phenotypic information in Fig. 3 into a single measurement that provides the most accurate possible prediction of the average performance of the offspring of the dog in question (Faera's Starlight). However, the individual's OFA page and vertical pedigree allows the breeder to determine where the liability comes from in the pedigree, the specific results from each mating and each dog's strengths and weaknesses. These are useful tools for selection and genetic improvement.

The distraction index (DI) measurement of the PennHIP method for hip dysplasia control employs a mechanical distraction device to measure maximal hip joint laxity as a predictor of future degenerative joint disease and osteoarthritis (Smith et al., 1990). PennHIP studies show that the OFA rating and DI measurement are significantly associated (Powers et al., 2010) and DI measurements submitted by their owners to the OFA are included in the hip dysplasia registry.

While the DI provides a measurement of laxity, it does not take into account degenerative joint disease or osteoarthritic changes. Studies have shown that liability for hip dysplasia and liability for osteoarthritis are controlled by separate genes (Clements et al., 2006; Zhou et al., 2010). The OFA hip rating incorporates an evaluation of both subluxation on the ventrodorsal hip-extended view, as well as radiographic anatomy and secondary boney changes.

The PennHIP method recommends selection based on the DI measurement of individual dogs. Based on PennHIP data of dogs presented to the University of Pennsylvania School of Veterinary Medicine, 100% of Golden retrievers and 89% of Labrador retrievers who received normal OFA ratings were deemed osteoarthritis-susceptible by their DI (Powers et al., 2010). Powers et al. (2010) also raised the possibility that the Cardigan Welsh Corgi is genetically fixed for hip dysplasia, based on DI measurements for the breed. However, the clinical presentation of disease in these breeds does not bear out these predictions, suggesting that there is a high false-positive rate for DI prediction of clinical disease. A study correlating ventrodorsal hip-extended radiographic ratings to later insurance-related claims for hip dysplasia showed a strong association (Malm et al., 2010). Data correlating DI measurements to morbidity from clinical disease have not been published.

Dog breeds have closed stud books and dog breeders have concerns about genetic diversity and the effects of artificial selection on their gene pools (Calboli et al., 2008). The removal of 89% or more of possible breeding stock for a single genetic disorder (which would be required in order to breed only from those Labrador retrievers with acceptable DI) will doom any breed to extinction from genetic depletion. While breeding from only OFA

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Excellent dogs will significantly improve hip ratings of progeny, the elimination of the rest of the phenotypically normal dogs from breeding (most of which produce predominantly normal dogs) would also severely restrict the gene pools of breeds. Pragmatic breeding recommendations include breeding from normal dogs with increasing normalcy of parents, grandparents, siblings and progeny, as shown on the OFA vertical pedigree, and through the use of EBVs.

The significant difference between progeny from one parent with normal elbows and progeny from two parents with dysplastic elbows suggests a qualitative trait. However, it is established that elbow dysplasia is a polygenic (multifactorial) trait (Engler et al., 2009). Increasing CPS tended to increase the frequency of elbow dysplasia in the progeny, but low numbers of submissions for some mating types between dysplastic parents skewed the results, making the correlation inconclusive. Again, pre-screening and non-submission to OFA of obviously dysplastic radiographs may have affected the data.

Grade I elbow dysplasia is a radiographic diagnosis that usually does not produce clinical disease or morbidity in the dog. Some breed groups counsel owners to ignore the diagnosis of Grade I elbow dysplasia and to treat these dogs as if they were normal. However, the data presented here demonstrates that progeny from a parent with Grade I elbow dysplasia, when bred to mates from all other rating classifications, have a significantly increased frequency of elbow dysplasia. These results are significantly different from the results observed with progeny from one normal parent bred to mates from all other rating classifications.

The data show that even two dogs with normal elbow radiographs may produce 10.1% progeny with elbow dysplasia. This is where consideration of depth and breadth of pedigree information becomes important. Any rating of elbow dysplasia in siblings of dogs with a normal elbow rating provides evidence that the normal dog may carry additional elbow dysplasia liability alleles.

Selection for increasing normalcy of depth and breadth of pedigree information provides a better selection tool for complexly inherited disease. The use of the OFA vertical pedigree provides the information necessary to make informed breeding decisions. The addition of EBVs that combine all of this information (Engler et al., 2009) and that also include genotypes of DNA markers for liability genes (Stock and Distl, 2010; Zhou et al., 2010) would be even more beneficial.

Conclusions

The OFA data show that hip and elbow conformation improve with improving parental phenotypic ratings. The open access OFA website provides health test results on individuals, as well as depth and breadth of pedigree health information on closely related individuals. This information provides the best means for making breeding decisions for both complexly inherited and Mendelian disorders.

Conflict of interest statement

The authors are Chief of Veterinary Services (GGK), Chief Operating Officer (ED) and Director (JSB) of the not-for-profit Orthopedic Foundation for Animals.

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References

AVMA Council on Veterinary Service, 1961. Report of panel on canine hip dysplasia. Journal of the American Veterinary Medical Association 139, 791–798.

Calboli, F.C., Sampson, J., Fretwell, N., Balding, D.J., 2008. Population structure and inbreeding from pedigree analysis of purebred dogs. Genetics 179, 593–601.

Clements, D.N., Carter, S.D., Innes, J.F., Ollier, W.E., 2006. Genetic basis of secondary osteoarthritis in dogs with joint dysplasia. American Journal of Veterinary Research 67, 909–918.

Corley, E., 1992. Role of the Orthopedic Foundation for Animals in the control of canine hip dysplasia. Veterinary Clinics of North America Small Animal Practice 22, 579–593.

Corley, E.A., Keller, G.G., Lattimer, J.C., Ellersieck, M.R., 1997. Reliability of early radiographic evaluations for canine hip dysplasia obtained from the standard ventrodorsal radiographic projection. Journal of the American Veterinary Medical Association 211, 1142–1146.

Engler, J., Hamann, H., Distl, O., 2009. Schätzung populationsgenetischer parameter für röntgenologische befunde der ellbogengelenkdysplasie beim Labrador retriever. Berliner und Münchener Tierärztliche Wochenschrift 122, 378–385.

Essman, S., Sherman, A., 2006. Comparison of digitized and conventional radiographic images for assessment of hip joint conformation of dogs. American Journal of Veterinary Research 67, 1546–1551.

Hou, Y., Wang, Y., Lust, G., Zhu, L., Zhang, Z., Todhunter, R.J., 2010. Retrospective analysis for genetic improvement of hip joints of cohort Labrador retrievers in the United States: 1970–2007. PLoS ONE 5, e9410.

International Elbow Working Group, 2001. 2001 International Elbow Protocol (Vancouver). www.iewg-vet.org/archive/protocol.htm (accessed 6 May 2011).

Keller, G.G., 2006. The Use of Health Databases and Selective Breeding: A Guide for Dog and Cat Breeders and Owners. Orthopedic Foundation for Animals, Columbia, Missouri, USA. www.offa.org/pdf/monograph2006web.pdf (accessed 6 May 2011).

Leighton, E.A., 1997. Genetics of canine hip dysplasia. Journal of the American Veterinary Medical Association 210, 1474–1479.

Malm, S., Fikse, F., Egenvall, A., Bonnett, B.N., Gunnarsson, L., Hedhammar, A., Strandberg, E., 2010. Association between radiographic assessment of hip status and subsequent incidence of veterinary care and mortality related to hip dysplasia in insured Swedish dogs. Preventive Veterinary Medicine 93, 222–232.

Paster, E.R., LaFond, E., Biery, D.N., Iriye, A., Gregor, T.P., Shofer, F.S., Smith, G.K., 2005. Estimates of prevalence of hip dysplasia in Golden retrievers and Rottweilers and the influence of bias on published prevalence figures. Journal of the American Veterinary Medical Association 226, 387–392.

Pirchner, F., 1983. Population Genetics in Animal Breeding, Second Ed. Plenum Press, New York, USA, 414 pp..

Powers, M.Y., Karbe, G.T., Gregor, T.P., McKelvie, P., Culp, W.T., Fordyce, H.H., Smith, G.K., 2010. Evaluation of the relationship between Orthopedic Foundation for Animals' hip joint scores and PennHIP distraction index values in dogs. Journal of the American Veterinary Medical Association 237, 532–541.

Reed, A.L., Keller, G.G., Vogt, D.W., Ellersieck, M.R., Corley, E.A., 2000. Effect of dam and sire qualitative hip conformation scores on progeny hip conformation. Journal of the American Veterinary Medical Association 217, 675–680.

Smith, G.K., Biery, D.N., Gregor, T.P., 1990. New concepts of coxofemoral joint stability and the development of a clinical stress-radiographic method for quantitating hip joint laxity in the dog. Journal of the American Veterinary Medical Association 196, 59–70.

Stock, K.F., Distl, O., 2010. Simulation study on the effects of excluding offspring information for genetic evaluation versus using genomic markers for selection in dog breeding. Journal of Animal Breeding and Genetics 127, 42–52.

Zhou, Z., Sheng, X., Zhang, Z., Zhao, K., Zhu, L., Guo, G., Friedenberg, S.G., Hunter, L.S., Vandenberg-Foels, W.S., Hornbuckle, W.E., Krotscheck, U., Corey, E., Moise, N.S., Dykes, N.L., Li, J., Xu, S., Du, L., Wang, Y., Sandler, J., Acland, G.M., Lust, G., Todhunter, R.J., 2010. Differential genetic regulation of canine hip dysplasia and osteoarthritis. PLoS ONE 5, e13219.

Zhu, L., Zhang, Z., Friedenberg, S., Jung, S.W., Phavaphutanon, J., Vernier-Singer, M., Corey, E., Mateescu, R., Dykes, N., Sandler, J., Acland, G., Lust, G., Todhunter, R., 2009. The long (and winding) road to gene discovery for canine hip dysplasia. The Veterinary Journal 181, 97–110.

The Canine Health Information Center, also known as CHIC, is a centralized canine health database jointly sponsored by the AKC Canine Health Foundation (CHF) and the Orthopedic Foundation for Animals (OFA). The program was originally conceptualized by the AKC Delegate Parent Club and Canine Health Committees. The CHF and OFA took on the project, and implemented it in the Fall of 2001 with eight pilot breeds participating. Today, nearly one third of the AKC Parent Clubs have joined CHIC, and nearly 20,000 dogs have met the individual breed health testing requirements and been assigned CHIC numbers.

The CHIC Mission Statement is quite simply "To provide a source of health information for owners, breeders, and scientists that will assist in breeding healthy dogs." The specific program goals are:

- To work with parent clubs in the identification of health issues for which a central information system should be established.
- To establish and maintain a central health information system in a manner that will support research into canine disease and provide health information to owners and breeders.
- To establish scientifically valid diagnostic criteria for the acceptance of information into the database.
- To base the availability of information on individually identified dogs at the consent of the owner.

The CHIC database is a tool that collects health information on individual animals from multiple sources. As more phenotypic and genetic screening tests become available and breeders make greater use of these tests, it is important that a database exists to capture this data. CHIC satisfies this need by functioning as a centralized pool of data. CHIC is about encouraging testing and health awareness and recording the results. It is not necessarily about 'normalcy' nor should it be misconstrued as an award program for normal dogs. Dogs with abnormal results are eligible for CHIC numbers as long as their results are in the public domain so that the breeders can benefit from the information in making more informed breeding decisions.

Core to the CHIC philosophy is the realization that each breed has different health concerns. Not all diseases have known modes of inheritance, nor do all diseases have screening tests. Some screening tests are based on phenotypic evaluation, others on genetic testing. With all these variables, a key element of CHIC is to customize or tailor the CHIC requirements to the needs of each breed. These unique requirements are established through input from the parent club prior to the breed's entry into the CHIC program. Breed specific requirements typically consist of the inherited diseases that are of the greatest concern and for which some screening test is available. Each parent club also drives specific screening protocols. As an example, one parent club may allow cardiac exams to be performed by a general practitioner. Another parent club may require the exam to be performed by a board certified cardiologist. A club may also use the CHIC program to maintain information on other health issues for anecdotal purposes. Later, as screening tests become available, the disease may be added to the breed specific requirements.

Regardless of breed, each dog must be permanently identified in order to have test results included in CHIC. Permanent identification may be in the form of microchip, or tattoo.

CHIC operates an informed consent database. All information regarding test results remains confidential unless the owner specifically authorizes release of the information into the public domain. Owners are encouraged to release all test results realizing it is in the ultimate health interests of the breed and the information greatly increases the depth and breadth of any resulting pedigree analysis. For those not quite ready to accept open sharing of information, there is still value in submitting their results. All test information entered into the database is available in aggregate for research and statistical reporting purposes, but does not disclose identification of individual dogs. This results in improved information on the prevalence of the disease, as well as information regarding progress in reducing the incidence of the disease.

A CHIC number is issued when test results are entered into the database satisfying each breed specific requirement, and when the owner of the dog has opted to release the results into the public domain. The CHIC number by itself does not imply normal test results, nor should it be interpreted as a 'stamp of approval" for breeding. The CHIC number only indicates that all the required breed specific tests were performed and the results made publicly available.

When a CHIC number is issued, a CHIC report is generated. The CHIC report is a consolidated listing of the tests performed, the age of the dog when the tests were performed, and the corresponding test results. As new results are recorded, updated CHIC reports reflecting the additional information are generated. For example, if a breed requires annual CERF examinations, an updated CHIC report will be generated every time updated CERF results are entered.

Once included in the CHIC program, the breed specific requirements are dynamic. As health priorities within a breed change, or as new screening tests become available, the breed specific requirements can be modified to reflect the current environment.

Health testing by itself is only the first step in attempting to reduce the incidence of genetic disease in our companion animals. It is important to take the next step and record the results in genetic health registries so that the data is preserved and others may benefit from it. Finally, the website brings things full circle by making the information easily accessible to the public via the internet. The CHIC website is located at www.caninehealthinfo.org. The website contains basic information on CHIC such as its mission and goals, and maintains a listing of the participating breeds and approved breed specific test protocols. The CHIC website also provides a search engine to locate dogs that have been issued CHIC numbers, their test dates, and the results of their tests. The website has been designed to seamlessly integrate with the existing OFA website.

The OFA and CHIC search engines allow queries to be very broad or very specific. Search criteria include registration numbers, registered names (including full name, first part of name, any part of name), breed, sex, birthdate (or range), specific disease registry, specific diagnostic rating, and report date (or range). Any combination of these search criteria can be specified resulting in a variety of potential matches.

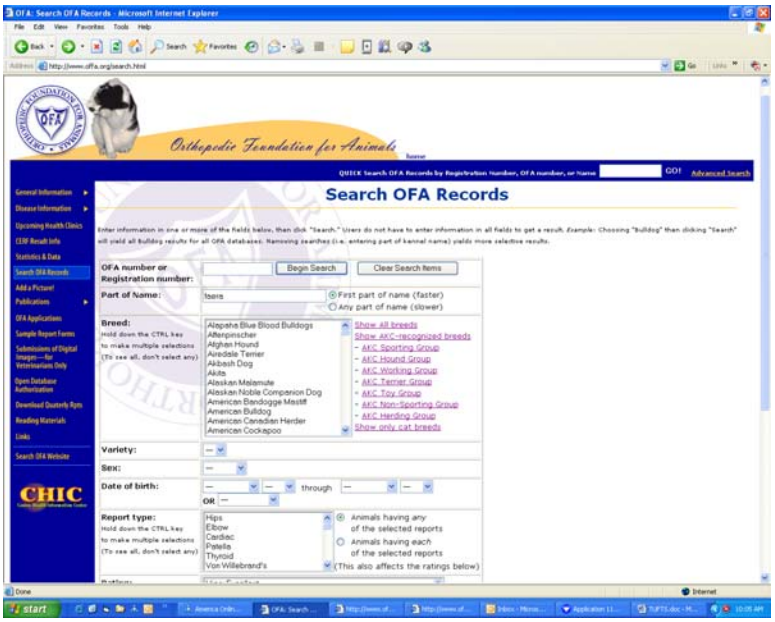

Figure 2, Screen Shot from the OFA website displaying the search criteria screen. In this example, the kennel name "Faera" was entered as the search criteria.

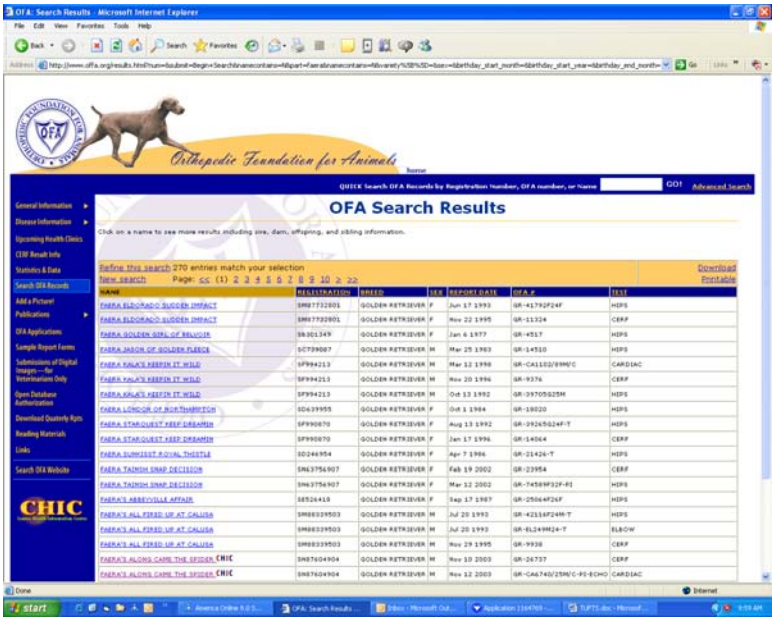

Figure 3, Screen Shot from the OFA website displaying the results using the search criteria of "Faera" in the name field.

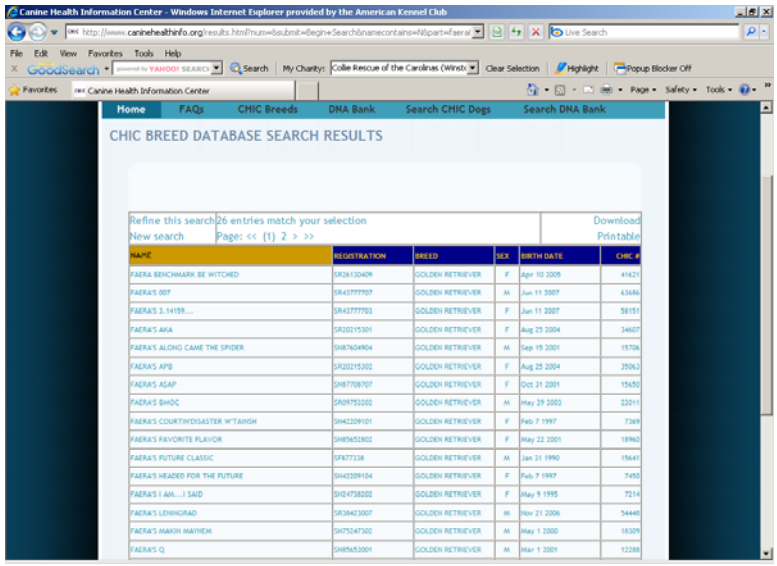

Figure 4, Screen Shot from the CHIC website displaying similar search results using the criteria of "Faera" in the name field.

Once an individual dog is selected through the search options, detailed information is displayed, including health screening done, age at the time of testing, and test results. In addition, wherever possible, the database does a pedigree query, and displays the dog's sire and dam, siblings (both full and half), offspring, as well as any of their recorded health test results. All displayed names are hotlinked, so the user can easily browse from dog to dog.

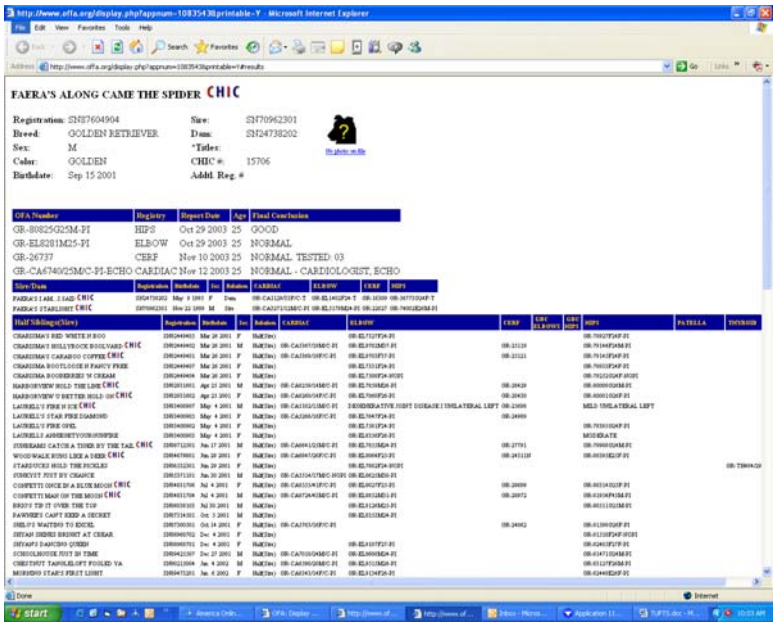

Figure 5, Screen Shot from the OFA website displaying individual dog record. Whether searching from the OFA site or the CHIC site, the individual dog records such as the one above are seamlessly accessed from either.

Another unique feature is the vertical pedigree analysis which encourages breeders to truly analyze the depth and breadth of a pedigree in a vertical fashion rather than the simple more traditional horizontal method.

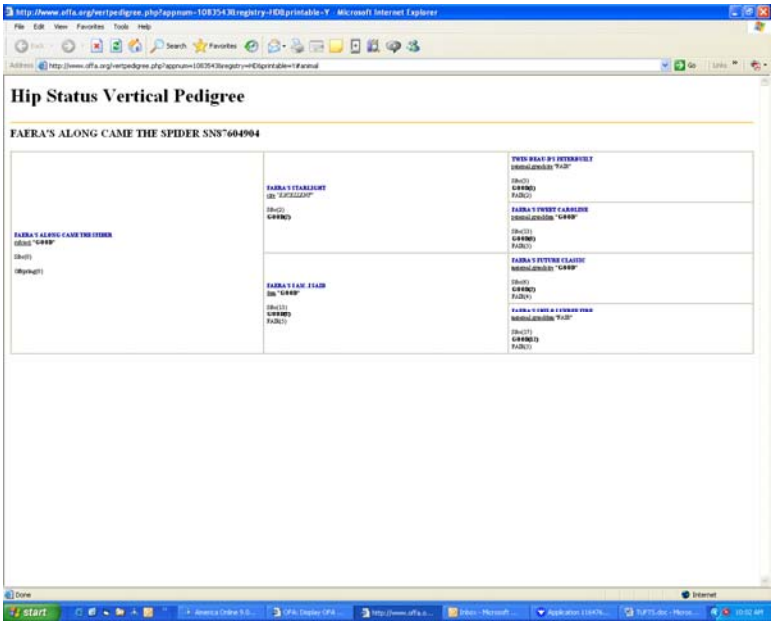

Figure 6, Screen Shot from the OFA website displaying vertical pedigree analysis information. Where available the pedigree shows not only the animals in the direct pedigree line, but summarizes information on sibling data.

The CHIC program offers a variety of benefits to breeders, buyers, parent clubs, and researchers. For breeders, CHIC provides a reliable source of information regarding dogs they may use in their breeding programs. In the future, breeders can begin to analyze the pedigrees of a proposed breeding for health strengths and weaknesses as well the traditional analysis of conformation, type, and performance strengths and weaknesses.

For buyers, the CHIC program provides accurate information about the results of a breeder's health testing. For diseases that are limited to phenotypic evaluations, there are no guarantees. However, the probability that an animal will develop an inherited disease is reduced when its ancestry has been tested normal. Further, as more DNA tests become available and the results are entered into CHIC, the CHIC database will help breeders predict whether progeny will be clear, carriers, or affected.

For parent clubs considering establishment of health databases on their own, CHIC provides the answer with no upfront investment required by the club. The CHIC infrastructure is supplied and maintained by the OFA. The data is maintained in a secure environment by trained staff. The services are not subject to the time, technology, and resource constraints that parent clubs might face on their own. This frees parent clubs to focus on the tasks of identifying health concerns, educating their membership, raising funds for research, and encouraging participation in the CHIC program.

For researchers, CHIC provides confidential and accurate aggregate information on multiple generations of dogs. CHIC information will also be useful for epidemiological studies enhancing our knowledge of health issues affecting all breeds of dogs.

For everyone interested in canine health issues, CHIC is a tool to monitor disease and measure progress.

The Orthopedic Foundation for Animals is a nonprofit 501(c)(3) foundation formed in 1966 with the following objectives:

- **To collate and disseminate information concerning orthopedic and genetic diseases of animals.**
- **To advise, encourage and establish control programs to lower the incidence of orthopedic and genetic diseases.**
- **To encourage and finance research in orthopedic and genetic disease in animals.**
- **To receive funds and make grants to carry out these objectives.**

The AKC Canine Health Foundation is a 501(c)(3) nonprofit organization formed in 1995 with the following mission: To develop significant resources for basic and applied health programs with emphasis on canine genetics to improve the quality of life for dogs and their owners. The AKC Canine Health Foundation is the largest funder of exclusively canine health research in the world.

The CHIC DNA Repository

jointly sponsored by the AKC CHF and the OFA

The CHIC DNA Repository is open to all participating CHIC breeds as of April 1, 2006. Our thanks to the Golden Retriever community for their overwhelming support of the project during the pilot phase!

MISSION

Jointly sponsored by the OFA and the AKC CHF, the CHIC DNA Repository collects and stores canine DNA samples along with corresponding pedigree and phenotypic health information to facilitate canine health research.

OBJECTIVES

- To facilitate more rapid research progress by expediting the sample collection process
- To provide researchers with optimized family groups
- To allow breeders to take advantage of future DNA based tests as they become available
- To foster a team environment between breeders and researchers, improving the likelihood of scientific discovery

SUBMISSION BY BLOOD SAMPLE

Blood is the gold standard for genetic material; the yield of DNA is sufficient for all research methods, including technologies on the horizon. Moreover, the stability and purity of the DNA is of the highest caliber, which offers many benefits. The drawback of banking blood samples is cost – drawing, shipping, storing, and extracting DNA from blood are more expensive endeavors than the alternative.

SUBMISSION BY CHEEK SWAB

Cheek swab-derived DNA is a viable option for DNA banking. Although the yield and purity of this DNA is inferior to that obtained from blood, the material is suitable for most genetic approaches. The swabs are inexpensive, and the samples can be taken by the owner of the dog without the necessity of a veterinary office call. Swabs are easily shipped in standard envelopes using the postal mail, and they can be stored for at least a decade at room temperature, so long as they are stored under conditions of low humidity. The success rate for obtaining DNA from a swab in the laboratory is roughly 98%, so multiple swabs should be submitted for each dog to ensure representation in the archive.

LABORATORIES

The CHIC DNA Repository has partnered wit the Veterinary Genetics Lab at the University of California-Davis and the Small Animal Molecular Genetics Lab at the University of Missouri. UC Davis receives and stores all swab samples, and Missouri receives and processes all blood samples.

PARTICIPATION

To participate, please visit the CHIC website at www.caninehealthinfo.org to download the DNA Bank application form. In addition to providing basic information on the dog, the owner will be asked to select their sample submission via blood or cheek swab. Once the application is processed, the owner will receive a swab or blood "kit" containing collection instructions, mailing labels, bar codes for the samples, and shipping labels.

Clubs may also contact CHIC directly at chic@offa.org if they are interested in setting up collection "clinics" at events such as shows or club meetings.

For more information email chic@offa.org
or visit the CHIC website at www.caninehealthinfo.org

Genetic Tests: Beyond the Basics

Danika Bannasch, DVM, PhD

The mode of inheritance of a particular disease is important to understand in order to interpret test results. The majority of DNA–based tests are for autosomal (not sex-linked) recessive disorders. If a disease is inherited as autosomal recessive, then the animal must have two copies of the mutant allele (version of the gene) to have the disease (genotype: d/d). If the animal only has one copy of the disease allele and the other copy is normal the animal will appear normal (genotype: d/N). Animals that have one normal allele and one mutant allele are called carriers. Identification of these animals is important to a breeding program since they appear completely normal but can produce affected offspring. If two carriers are bred to each other 25% of the offspring will be affected with the disease (d/N X d/N results in 25% d/d, 50% d/N, and 25% N/N- see Table 1).

Table 1: Simple recessive disease

	d	N
d	**d/d**	**d/N**
N	**d/N**	**N/N**

Table 2: Genotype - disease correlation simple recessive

Genotype	Disease state
d/d	**100% Diseased**
d/N and N/N	**Normal**

d= disease allele N = the normal allele

Breeders can utilize the DNA tests to identify carriers and avoid breeding them to each other. The simple recessive mode of inheritance underlies the majority of DNA based tests available to date; however, there are notable exceptions and, in the near future, more exceptions are likely to be made available to breeders. This article will focus on understanding these exceptions.

Some diseases are not inherited in a simple manner. Some are caused by mutations in multiple genes or a combination of gene and environment interaction while other disease may have unidentified complexity. This does not imply that there is not an underlying genetic component that can be tested and used for breeding but the relationship of genotype to disease is not as straightforward as in the example above. To understand these more complicated aspects, let's first consider some variations on our original simple autosomal recessive mode of inheritance. There are still two copies of every gene in every dog so, from the testing standpoint, the results reported will be the same. The difference lies in the fact that not all d/d animals will get the disease. This can occur for many different reasons and those reasons may be labeled differently by geneticists.

The first reason that there might not be a one to one relationship between the genotype and the disease state is reduced penetrance. The term reduced penetrance just means that rather than 100% of the d/d animals getting the disease less than 100% will develop it. If a disease is reported to have reduced penetrance, then the amount should be reported - even if it is just an estimate. For example, a reduced penetrance of 85% means that 85% of the d/d animals will get the disease. A penetrance of 5% means that only 5% of the d/d animals will get the disease. One key point about a disease with reduced penetrance is that an animal that has the d/d genotype may be clinically normal and therefore might have been used for breeding with the assumption that the animal was either a carrier or even clear. Therefore in cases where a genetic disease has reduced penetrance, a DNA test is useful to identify carriers (d/N), normal animals (N/N) and normal animals with the disease genotype (d/d) prior to breeding.

Table 2: Genotype - disease correlation for a disease with 20% penetrance

Genotype	Disease state
d/d	**20% Diseased, 80% Normal**
d/N and N/N	**Normal**

The second reason that not all d/d animals will get the disease is if the gene is a susceptibility gene. In this case, it could be dominant or recessive but it confers susceptibility to disease. The DNA test interpretation requires knowing what percentage of animals with the susceptibility allele will get the disease. This is called the relative risk. Animals with two copies of the risk allele may be more likely to get the disease than animals with one copy of the risk allele. The inheritance and ratios will be the same as in table 1 but the genotype- disease correlation will be different. An example of a susceptibility gene with moderate risk is shown in Table 3.

Table 3: Genotype - disease correlation for a disease with a moderate relative risk

Genotype	Disease state
r/r	5 times more likely to get the disease than N/N
r/N	2.5 times more likely to get the disease than N/N
N/N	No increased risk

Both of the previous examples can be confounded by phenocopies or molecular heterogeneity. A phenocopy is a disease state caused by an environmental factor and molecular heterogeneity means that there is more than one genetic cause of the same disease. The DNA based tests are ONLY testing for the specific gene and allele that they have been designed for - not every possible cause of the same disease.

There are some general principals of DNA based genetic tests that researchers can use to evaluate their potential disease causing mutations. Within the breed where the test was developed, are all affected animals explained by mutation (heterogeneity)? This percentage should be as close to 100% as possible. What percentage of unaffected animals has the mutation (penetrance or risk)? This percentage should be as close to 0% as possible. As these two percentages fall away from the ideal the test becomes more and more suspect and less useful as a tool to decrease the incidence of diseased puppies.

One last confounding factor is the use of linked marker tests where the disease mutation is unknown but a marker nearby is used to reflect the state of the actual mutation. There are error rates associated with these types of tests because sometimes the marker does not reflect the actual disease allele. Linked marker tests can be used within families to determine disease states but there can be problems using them in unrelated individuals. The reason that linked marker tests or haplotype tests would be used is the long period of time it can take researchers to find a causative mutation. In the meantime, in order to assist breeders, a test will sometimes be offered that may be imperfect.

While this may all seem very complicated, the good news is that tests are available that can help breeders decrease the risk of producing affected dogs. However, caution should be used in aggressively selecting against mutant alleles which have low penetrance or low risk for the disease state when the mutant alleles are common throughout the breed. This could result in a reduced gene pool and reduced genetic heterozygosity leading to other potential health risks.

Cancer 101

This information and much more is available by the Colorado State University - Animal Cancer Center and can be found at: www.csuanimalcancercenter.org

About Cancer in Pets

There are three basic steps in cancer causation. These steps ultimately lead to the evolution of a normal cell to a cancer cell.

- Initiation: Initiating agents induce a permanent and irreversible change in the DNA of the affected cell. In and of itself, the initiating event is not significant enough to induce cancer transformation.
- Promotion: Promoting agents cause reversible tissue and cellular changes. Promoting agents can result in changes in the shape of a cell, its growth rate, and degree of differentiation. Promotion serves to expand the initiated cell population and alter it in such a way as to increase the likelihood of cancer.
- Progression: Progressing agents are able to convert an initiated cell, or a cell undergoing promotion, into a cell exhibiting malignancy.

In order for a tumor to result, the affected cell must be irreversibly altered at least twice. The cell is altered once in the initiation phase and once in the progression phase. The promotion phase changes the affected cell in a way to increase the likelihood that the cell changed by the initiation will be in a position to be changed by the progression phase.

Cancer is a common and serious disease. Many owners have had or will have personal experience with cancer in themselves, a family member, or a close friend. Keeping this in mind, we should approach the pet with cancer in an educated, positive, and compassionate manner. With increased optimistic media coverage, pet owners are becoming more knowledgeable and more proactive in seeking care for their pets with cancer. When clients hear about advances in human medicine, they expect the same treatment options for their pets.

A Few Cancer Facts:

- Cancer is the uncontrolled growth of abnormal cells on or within the body. Not all cancers are the same. Depending on the location and biologic behavior there may be several treatment options available.
- Cancer is the leading cause of death in pet cats and dogs in the United States. As many as 50% of pets die of cancer.
- Cancer is often a treatable or even curable disease with specialized cancer care.
- Many of the same treatments that are available for humans are now available for pets. These treatments include chemotherapy, radiation therapy and surgery.
- Different cancers may require different forms of treatment. Some patients will only need tumor removal where as others may need a combination of treatments.

Top Tens Warning Signs of Cancer in Pet Animals

1. Abnormal swellings that persist or continue to grow
    - Pet your pet! This is the best way to find lumps, bumps or swellings that could be anywhere on the body.

2. Sores that do not heal
    - Non-healing sores can be a sign of infection or cancer. Your veterinarian can determine the reason why the sore is not healing.

3. Weight loss
    - If your pet is not on a diet but is losing weight, illness could be to blame.

4. Loss of appetite
    - It is not normal for pets to lose their appetite. This may be a sign of illness.

5. Bleeding or discharge from any body opening
    - Bleeding can occur for numerous reasons - most of which are abnormal. Vomiting and diarrhea are abnormal discharges as well!

6. Offensive odor
    - This is a common sign especially for tumors in the mouth, nose or anus.

7. Difficulty eating or swallowing
    - This is a common sign of cancers of the mouth and neck region.

8. Hesitation to exercise or loss of stamina
    - This can be one of the first signs that your pet is not feeling well.

9. Persistent lameness
    - There could be many causes of lameness including nerve, muscle or bone cancer.

10. Difficulty breathing, urinating or defecating
    - If your pet experiences any of these symptoms please have them evaluated by a veterinarian.

Useful Links - Canine Health Research

Check out the AKC Canine Health Foundation website www.akcckhf.org. There are several resources available for you but here are a few highlighted for quick reference:

Research Participation www.akcchf.org/research/participation-needed
(Sample Collection and Clinical Trials)

Searchable Genetic Tests www.akcchf.org/canine-health/genetic-tests

Grant Sponsorships www.akcchf.org/sponsor

Research Success Stories www.akcchf.org/research/success-stories

Glossary of Terms www.akcchf.org/canine-health/glossary

Educational Podcasts and Videos www.akcchf.org/news-events/multimedia
(Including NPCCHC Presentations)

And Many More!

And here are a few additional Websites for Canine Health:

Orthopedic Foundation for Animals www.offa.org

Canine Health Information Center www.caninehealthinfo.org

Canine Comparative Oncology and Genomics Consortium (CCOGC) www.ccogc.net

Canine Hereditary Cancer Consortium www.vai.org/helpingdogs

Canine Genetic Diseases Network www.caninegeneticdiseases.net

Colorado State University-Animal Cancer Center www.csuanimalcancercenter.org

The Penn Vet Working Dog Center is excited to announce the **2011 Working Dog Conference** "Defining, developing, and documenting success in working dogs". This conference will be held September 7-9, 2011 in Pearl River, New York in conjunction with Finding One Another: 10th Anniversary Tribute to the 9/11 Canine Search & Rescue Community (www.FindingOneAnother.org).

The mission of the Penn Vet Working Dog Conference is to foster the exchange of knowledge and ideas that will drive advancements in the care and training of the working dog. This conference will draw over 200 canine handlers, veterinarians, breeders, trainers, and researchers representing working dogs in the fields of Seeing Eye, Search and Rescue, Narcotics/Explosive Detection, Homeland Security and more. With attendance drawing from across the United States and internationally, attendees will be able to interact with key members of the working dog community. Presenters include Scott Thomas from the TSA Breeding Program, The Seeing Eye Director of Canine Development-Peggy Gibbon, and the pioneering founder of Puppies Behind Bars-Gloria Gilbert Stoga. We will also have presenters from over five countries including researchers from the University of Sydney and University of Bristol.

Hope to see you there.

Visit www.PennVetWDC.org or http://www.findingoneanother.org/upenn-registration to register and see complete speaker program and event schedule.

AKC Canine Health Foundation is a Proud Supporter of this Conference.

Thank You!

Conference Co-Chairs:

Lee Arnold
Steve Remspecher

Conference Sponsor:

PURINA

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Ms. Connie G. Miller
Mr. Richard Nance

Ms. Nancy Simpson
Mr. Charles Teasley
Dr. and Mrs. William Truesdale
Mrs. Cindy Vogels

And a very special thank you to all the participants and clubs who have helped make this program thrive over the years. Without your valuable support, AKC the Canine Health Foundation would not be as successful at helping all dogs lead longer, happier, healthier lives.