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Day One: Welcome

Lee Arnold
Secretary
AKC Canine Health Foundation (CHF)

Deborah (DD) DiLalla
Executive Director
AKC CHF

Lee Arnold said it was exciting to be in a room “full of folks who are so energized, dedicated, and committed to finding a cure to those diseases that affect all of our dogs.” He noted that the AKC Canine Health Foundation (CHF) has emerged over the last 13 years as the world’s pre-eminent leader in funding canine health research, with more than \$20 million invested in projects “that have benefited both our canine and our human neighbors.”

The canine genome was released in December 2005, just after the last CHF conference. “Since then, we’ve seen remarkable success and enormous discoveries,” Arnold said. “The genome has really become a very powerful tool in the fight against canine illness, and since canines share much of our genetic makeup, the crossover to human health research is extraordinary.”

The result, he said, is that “man’s best friend is really becoming our best friend in the fight against disease.” Arnold thanked the AKC for its ongoing support and Nestlé Purina PetCare Company for sponsoring the conference.

Deborah DiLalla introduced CHF staff and acknowledged the 18 months of effort that the Foundation and Nestlé Purina had devoted to organizing the conference.

The Human/Canine Connection

Matthew Breen, Ph.D.
North Carolina State University

The release of the canine genome sequence has brought more attention to dogs as a “powerful biomedical model,” Dr. Breen said. One result has been a larger number of people asking for a definition of a domestic dog.

Citing a passage from Rudyard Kipling’s *Just So Stories*, Dr. Breen said the relationship between humans and canines can be traced back 30,000 to 50,000 years. Some of the earliest surviving records of that relationship date back to ancient Egypt, where pictographs on gravestones and coffins showed different dog breeds associating with members of the nobility.

Dr. Breen showed a print from the world's first official dog show, conducted by the Kennel Club of England in 1873. "We can now start to see that many of the dogs that were around did not resemble the ancient breeds," he said. "Instead of breeding dogs primarily for their function, we started to breed for their phenotype," as a growing leisure class began inbreeding and linebreeding for specific characteristics that they favored.

The bond between dogs and humans "really took force when dogs became members not only of our households, but of our families," Dr. Breen said. Dog owners take their companions everywhere. Dogs are trained to understand humans, and many still serve their owners as intruder alarms, as they have over the millennia.

Dog owners have joked for many years about their physical similarities to their pets. With the release of the canine genome sequence, there is now evidence that dogs and humans are 85%–100% similar at the genetic level.

"My perception as a geneticist is that we're all just mammals, and as mammals we're all just differential rearrangements of the same collection of ancestrally related genes," Dr. Breen said. "We all have the same genes, and we all have about 20,000 of them," so the only difference is in the way the genes interact and are expressed.

Part of what makes canine research powerful is the potential for "multiple generations on the ground at the same time. We can't do that for humans." An important consequence of linebreeding is that the same ancestors appear multiple times in the same purebred pedigree, often showing up in both the maternal and paternal line.

"It's not surprising that our dogs are now being described in a politically correct way as 'genetically challenged,'" with many breeds facing a serious reduction in their genetic variability. High levels of inbreeding lead to smaller litters and reduced fecundity, outcomes that Dr. Breen described as nature's way of slowing down the process. As researchers, veterinarians, breeders, and owners all learn how to prolong life and health, the dogs breed more, and genetic problems can be perpetuated.

After 300–400 years of selective breeding practices and severely reduced variability, dogs are presenting with genetic diseases that affect every body system. Dr. Breen said 25% of purebreds in the United States are either affected by or carriers of a serious genetic disease. By contrast, in the much more genetically diverse human population, a 1% risk of genetic disease is considered shocking and worthy of medical attention.

"This is why I always like to regard the canine genome as man's new best friend," Dr. Breen said. He cited narcolepsy and Lafora's disease, one of five known inherited progressive epilepsy syndromes, as examples of human conditions better understood as a result of canine genetic research. Researchers working with the Doberman isolated the narcolepsy gene and are testing a therapy that could help 250,000 Americans if it proves to be effective on dogs.

In Dr. Breen's specialty area, canine cancer, "we have shown quite irrefutably that dogs and humans present with the same chromosome aberrations, the same genetic lesions, in corresponding cancers." He said he has spoken to human cytogeneticists who initially questioned the genetic link; after checking further, they reported back that the anomaly was very rare. "That's because the technology is just not capable of finding these aberrations in humans."

After coexisting with dogs for tens of thousands of years, Dr. Breen said humans have two good reasons to study canine genetic disease. The first is that breeding programs are responsible for many canine diseases. The second is that canine research can help health researchers understand human biogenetic disease. "The irony is that the keys to unlocking these intriguing puzzles may be sitting, walking, and sleeping right beside us," so that the emotional bond between dogs and humans "is ever so much fortified by the biomedical relationship that we share."

Discussion

A participant said more research on specific targets would be needed before human gene therapies can be adapted for use with canine cancer patients. Dr. Breen said a key challenge for canine health research is to demonstrate a strong enough biomedical relationship between humans and dogs to "raise eyebrows with Big Pharma and make them interested."

Dr. Breen agreed with the participant's statement that researchers need more information on biology. "That's why I wholeheartedly support the mission of CHF, because I don't have to justify the dog as a model," he said. "I've gone on record that studying dog cancers over the next five to 10 years will yield more cancer-associated genes than working in the same field in humans," thereby benefiting dogs first before the results move back to humans.

At another participant's invitation, Dr. Breen announced that he and Dr. Jaime Modiano had just secured a five-year, \$1-million grant from the US National Institutes of Health (NIH) to study lymphoma genes in dogs and associate the results back to human cancer research. "NIH doesn't fund projects unless they think they're going to win," he said. A five-year grant in tight financial times is a testament to CHF's foresight in funding the initial research that led to the grant.

A participant recalled researchers' commitment in the mid-1960s to "conquer cancer" within 10 years. At the time, he said, NIH scientists saw no value in dogs as a model for understanding human cancer. Now, "we may be able to go light years ahead in understanding human cancer by understanding dog cancer."

A participant emphasized the genetic damage that results when popular sires are overused in breeding programs. "People should go back and point this out to their kennel clubs," Dr. Breen

said. “The damage is unbelievable, and you won’t know about it for two or three generations, until these dogs start crossing back” through the pedigrees.

Genetics Primer

Anita Oberbauer, Ph.D.
University of California, Davis

Jerold Bell, DVM
Tufts University

Dogs are the most genetically engineered species on the planet, said Dr. Anita Oberbauer. This reflects breeders’ efforts to maximize or minimize specific heritable traits. As far back as ancient Egypt, dog owners would “try and breed the best to the best,” but the outcomes were based on likelihood and probability. Genetic tests have introduced more certainty into the selection process.

Dr. Oberbauer gave participants an overview of the terminology and dimensions of canine genetics:

- An animal’s appearance is its *phenotype*. Its genetic characteristics are its *genotype*.
- The animal’s basic genetic characteristics are carried in its DNA located in the nucleus of the cell. Each strand of DNA is made up of *nucleotide bases*—labeled A, C, G, and T—that combine into the template for a gene.
- *Genes* are regions on a DNA strand governing a particular aspect of the genotype, such as hair length. A gene is the blueprint for a protein.
- The DNA in the nucleus is made into *RNA*, which is then translated into protein.
- Canines have just over two billion nucleotide bases, translating into about 20,000 unique genes. The genes are packaged into 38 DNA regions, called *chromosomes*. Every dog has 38 pairs of chromosomes, called *autosomes*, as well as the XX or XY chromosomes that govern gender.
- Chromosomes come in pairs, and the two *copies* of each gene are called *alleles*. The underlying DNA governs differences between the alleles. Each pair of alleles is called a *diploid*, and each one governs a specific genetic trait, like growth, fat, or hair color.

The location of each diploid on a chromosome is the identifying address for that gene, known as the *locus*, and the locus is always the same; for example, the gene for von Willebrand’s disease (vWD) is always in the same location on chromosome 10 in dogs. Humans have a comparable genetic structure, but the addresses often differ. vWD, for example, shows up on chromosome 12 in humans.

Changes or mutations in a breed's DNA may or may not be favored by breeders and may be positive, negative, or neutral for the health of the animal. Although an individual dog can only have two alleles in each chromosomal pair, there may be many different alleles across a breed population.

Alleles can either be identical (*homozygous*) or different (*heterozygous*), and may be dominant or recessive compared to other alleles in the breed population. In Labrador Retrievers, for example, black hair is dominant and brown hair is recessive, so a chocolate Labrador only results from the combination of two recessive genes. In a combination of dominant and recessive alleles, the black trait would mask the brown, although the dog would still carry the recessive gene.

The Merle gene in the Shetland Sheepdog is an example of incomplete dominance of one allele over another. While the classic Merle coloring results from a heterozygous pair, the combination of two dominant Merle alleles results in white patching and a number of serious health issues for the dog.

The essence of a breeding program is that the offspring take half their genetic material from each parent. Through the process of *meiosis*, one allele from each pair is selected and recombined with one corresponding allele from the other parent. The separation process is governed by Mendel's Law of Segregation, which holds that offspring only receive one copy of each paired chromosome from each parent, and Mendel's Law of Independent Assortment, which reflects the random way in which genes are selected.

Dr. Oberbauer said the structure of genes allows for a great deal of crossover, i.e. recombination, during meiosis. Others are packed more closely together on a chromosome, so that the alleles of two linked genes are more likely to travel together. These linked alleles are called *haplotypes*.

Some diseases are genetic but not inherited, she said. A chemically induced leukemia or a developmental anomaly might change an animal's DNA, but "it's not in the sperm, it's not in the egg, and it will not be passed on to the next generation." If a trait is passed on, it is important to estimate the degree of its heritability and determine whether it is regulated by one, two, or several genes. These factors "determine how well you can make genetic progress in engineering your animal."

Some traits are also influenced by sex. With one X and one Y chromosome, the male determines the sex of the offspring, but might also pass on sex-linked traits like hemophilia in humans or calico markings in cats. Some traits are also *polygenic*, or complex, meaning that both parents contribute alleles that influence their expression.

Dr. Oberbauer identified inbreeding, linebreeding, phenotypic breeding, outcross breeding, and compensatory breeding as the main strategies for encouraging or discouraging specific genetic

traits. Inbreeding and linebreeding promote uniformity — “that’s how breeds are created,” she said. By narrowing the gene pool and increasing the prevalence of recessive traits, the practices can also make deleterious alleles more visible in a breed population. If fewer alleles are present and one of them happens to be defective, “you basically have that allele in your population.” This is particularly true when a “popular sire” is overbred.

Phenotypic breeding focuses more on a dog’s appearance and less on its pedigree, but Dr. Oberbauer said the larger range of possible allele combinations makes it less likely that specific traits will be passed on to offspring. Outcrossing is a method of improving a breed, by introducing heterozygosity and compensating for a deleterious recessive allele. Compensatory breeding is used over a period of generations to correct an obvious phenotypic fault.

The ultimate goal is to combine genetic knowledge with breeding strategies that address the risk of disease or the likelihood of repeating a desirable trait. Dr. Oberbauer emphasized the need to prioritize traits, suggesting that a dog testing positive to develop cataracts late in life might still have enough positive characteristics to be bred. Over the longer term, new genetic technologies will challenge breeders to “make more distinct and difficult choices,” while still setting out to breed the best of the best.

Noting that he sees genetic disease in a large proportion of his patients, Dr. Jerold Bell emphasized the need to breed responsibly and identify diseases that can be reduced through careful selection. He said the preponderance of genetic disease traces back in part to changes in breeding practices over the past century.

“Old-time breeders spent a lot of time working on breed histories, and understanding selection parameters for their particular breeds and the breed characteristics that should be selected for,” he said. For working breeds, selection was as simple as not breeding a dog that could not perform its function. “Their genetic testing was seeing a hunting dog that couldn’t hunt, or didn’t have the right temperament.” Today, many more people are breeding dogs, and much less time is spent scrutinizing each breeding decision.

The latest development is the introduction of so-called “designer breeds.” “The majority of new puppies coming into my practice for the past two years are designer-bred dogs, not purebred dogs,” Dr. Bell said. “The fraud being perpetrated on the American public is that somehow these dogs are going to be healthier than purebred dogs,” when “the only way we can ensure the increased health of the offspring is through genetic testing and the selection of healthy parents.”

Dr. Bell said he sees as much genetic disease in mixed-breed dogs as in purebreds and designer breeds, if not more. The difference with purebreds is that breeders undertake genetic testing and “scream out loud” if something is wrong. One study of canine hypothyroidism found an average rate of 7.5% across all AKC breeds and 10.7% in mixed breeds, based on a pool of more than 55,000 samples. The results may have been skewed, since mixed breeds were only tested

based on clinical signs. "But it tells us that this disease is present across all dog types," showing that the cause is probably an ancient gene inherited by multiple breeds.

For the 10 top diseases identified in CHF's 2002 and 2004 breed health surveys, Dr. Bell said rates are often far lower in purebreds based on genetic selection than in the general canine population.

The availability of genetic testing means taking a second look at the definition of a reputable breeder. "The times have now changed," Dr. Bell said. "It's not a crap shoot anymore. It's still a roll of the dice, but the dice are now loaded." As custodians of their breeds, reputable breeders must take advantage of genetic testing to deliver a healthier product to the American public.

Gene frequency is not altered by breeding practices, he said, but by selection. With a finite number of quality dams, overbreeding of popular sires prevents other males from contributing to the gene pool, and genetic diversity suffers.

Managing genetic disease means relying on genetic test results and registries to confirm genotype, since phenotypic linkage tests can yield false positive and negatives. When a test is available for carriers, a breeder has the option of breeding a quality carrier to a genetically normal mate, then replacing the quality parent with a quality, genetically normal offspring. This practice reduces the frequency of the defective gene with each generation, and eventually eliminates it.

By the same token, Dr. Bell warned that genetic testing could be the Trojan horse that devastates a breed. The worst decision a breeder can make is to refrain from breeding a favored dog because of one genetic test result, he said. "You've already decided it was a quality animal, and a single testable gene result should not alter the decision about an entire animal" that has 20,000 to 30,000 genes.

Without a test for carriers, the only option is to breed higher- and lower-risk individuals and continue replacing the quality parents with lower-risk offspring, a practice that depends on open health databases, registries, and open communication among breeders. Dr. Bell identified the Canine Eye Registry Foundation (CERF), the Orthopedic Foundation for Animals (OFA), and the Canine Health Information Center (CHIC) as powerful resources for both veterinarians and breeders.

The databases can also be used to educate new owners about the characteristics of their breeds. "I don't know how else to do it except one owner at a time, to educate the public that they need to do as much research when they buy a puppy as when they buy a refrigerator."

At a time when no dog is likely to be perfect, the databases become a tool for promoting health consciousness, not health normalcy. "As long as we keep the problems secret, we will not be able to deal with them," Dr. Bell said. "We here represent every AKC breed, and we need to go

forth and pass the word that this is a new age. It's different now, and this is the way we go forth."

Understanding How Breed Relationships Facilitate Genetic Studies of Complex Traits and Diseases

Heidi Parker, Ph.D.
National Human Genome Research Institute
National Institutes of Health

Dr. Heidi Parker said there are about 400 breeds of domestic dogs worldwide, representing the greatest diversity in size, shape, and behavior of any mammalian species. Some breeds are more than 1,000 years old, others are less than 100 years old, but all are characterized by phenotypes, such as how they look and how they act.

She described the "Phy-Do" (Phylogeny of the Dog) study, which helps show what DNA can reveal about the relationships between breeds, and how researchers can use that information to help find genes that cause particular traits or diseases.

The Phy-Do study examined 132 breed variants, looking at 96 genetic markers spread across 38 autosomes, and showed that every breed has a different specific genetic signature. To determine how the breeds relate to each other, information from 628 dogs representing those 132 breeds was divided into five clusters using only genetic data.

- Asian breeds, including Akitas, Shar-Peis, Siberian Huskies and Alaskan Malamutes, form the first cluster. When wolves were added to the analysis, they grouped with this cluster. This suggested that they could be either the oldest identified specific breed of dogs, relating back to their ancestors, or the most recent.
- The second cluster comprises the Mastiff group, including Mastiffs, Bulldogs, Bull Terriers, and some other Terrier relatives.
- Herding dogs, such as Collies, Greyhounds, and Whippets, form the third cluster.
- The fourth and largest cluster is modern or hunting dogs, including Pointers, Setters, a number of the Retrievers, and a subset of the Spaniels. Most, but not all, sporting dogs ended up in this group.
- Mountain dogs, such as Bernese Mountain Dogs, Saint Bernards, German Shepherds, the rest of the Spaniels, and the Standard Poodle form the fifth cluster.
- A sixth group includes miscellaneous dogs, including toy breeds that show some relationship to each other and to sporting dogs, herding dogs, and small Terriers.

This information can be used to understand how these dogs are related to each other, Dr. Parker said, and to determine the origin of a gene that causes either a morphological trait or a disease,

as well as what other breeds might share it. For example, if two closely related breeds share a disease, it is possible to include individuals from both groups when trying to map it. This gives researchers a larger sample set to help identify the gene that causes the disease.

Distantly related breeds that do not share all of their genetic information, but do share the same disease, can be used to reduce the region of linkage. "When we've linked something to a section of a chromosome, we can combine those breeds together to find that actual small region that contains the gene of interest," Dr. Parker said.

Unrelated breeds with similar traits and similar phenotypes of disease, without inheriting a disease from the same ancestors, can suggest which genes are in the same pathway and which genes are interacting, she said. This tells researchers more about the disease and possible treatments for it.

Closely related dogs that share traits without sharing a disease are useful in ruling out false positives, and identifying breed-related information pertaining only to a specific disease.

Dr. Parker described how this methodology has been applied to a particular disease to find the mutation that caused it.

One study dealt with Collie eye anomaly (CEA), a developmental defect that results in choroidal hypoplasia and a pale patch in the back of the retina. Using a set of 14 families, Dr. Parker said, this disease was mapped to a region on chromosome 37 that was about three centimorgans long and included up to 40 genes.

To narrow this large region down to a smaller number of genes, researchers looked at affected dogs from four related breeds—Collie, Shetland Sheepdog, Australian Shepherd and Border Collie—and found a section of about 100,000 bases (100 KB) that all four had in common. This section was not found in unaffected dogs, and all affected dogs had at least one copy of this particular sequence. Dr. Parker said this was "exactly what we were looking for." Researchers then sequenced the smaller region, which included only four genes instead of 40, and were able to identify the specific mutation associated with CEA. The information was used to develop a test for the disease.

After finding the mutation, researchers looked at a number of other breeds that share the same phenotype, finding the same mutation in Lancashire Heelers, Long-Coated Whippets, Nova Scotia Duck Tolling Retrievers and Boykin Spaniels. All carried the same disease gene, which Dr. Parker said was probably inherited from a Collie-type ancestor.

Another study looked at dogs with Addison's Disease, a primary adrenocortical insufficiency that is more complex than CEA. Addison's Disease causes skeletal muscle atrophy and a shortened life span, even with treatment. Research into the human form of the disease suggests that it is autoimmune and polygenic, meaning that many genes, as well as some environmental

factors, are involved in causing the disease. Both make it difficult to identify the specific genes involved.

Starting with a group of more than 10,000 dogs, Dr. Parker said researchers identified 166 affected Portuguese Water Dogs, of which 78 were still alive and viable for participation in genetic studies. Using a genome scan, researchers identified two loci for Addison's Disease, at CFA 12 and CFA 37. One seemed to give an increased risk for Addison's Disease, while the other decreased it.

To help identify the specific genes responsible in each region, researchers examined a group of Nova Scotia Duck Tolling Retrievers, another breed prone to developing Addison's Disease. This group included 15 unaffected dogs and 14 affected dogs, Dr. Parker said.

In the Portuguese Water Dogs, the study had found three genes on chromosome 12 that were highly associated with Addison's Disease. In the Nova Scotia Duck Tolling Retrievers, Dr. Parker said, only one of the three genes appeared to be associated.

A similar approach was used in looking at chromosome 37, where researchers found two peaks of association in Portuguese Water Dogs. When the Nova Scotia Duck Tolling Retrievers were added to the study, researchers were able to discern between the two peaks to identify the one most likely to be associated with the disease.

A third study dealt with malignant histiocytosis (MH), a blood-borne cancer, in Bernese Mountain Dogs. This population study examined 500 microsatellites on 175 dogs—55 with MH and 120 controls—and compared the data to a family study done simultaneously in France that included 260 microsatellites and about 92 individuals, all from one large family. Researchers found at least two loci in common between these two groups: one was on chromosome 8 and the other on chromosome 20. To narrow down the region even more, researchers plan to compare the results to those from a group of Bernese Mountain Dogs in Holland, and to the Greater Swiss Mountain Dog, a closely related breed that does not have the same high incidence of malignant histiocytosis.

Dr. Parker noted that, unlike family studies, population studies offer the opportunity to map a specific chromosome or region within one generation, comparing affected dogs to unaffected ones. In family studies, researchers must go back to the parents and grandparents to determine what is inherited and how it is passed down through that family. This can be difficult to do, especially with late-onset diseases. By the time an adult dog develops such a disease, its parents and grandparents may have already died. Another advantage of population studies is that the data sets can be used for multiple studies looking at different diseases or traits, Dr. Parker said.

Canine Reproduction

Margaret V. Root-Kustritz, DVM, Ph.D., DACT
University of Minnesota

Dr. Margaret Root-Kustritz said that gonadectomy, the standard practice for sterilization of dogs and cats in the United States, has traditionally been done around the age of six months. Though the procedure is commonly performed, there is uncertainty about whether elective sterilization is beneficial, and if so, whether six months is the optimal age to spay or neuter a dog.

A wide range of benefits and detriments are associated with sterilization. "I'm not going to tell you this is the age you should spay or neuter," Dr. Root-Kustritz said. The decision should be based on the breed, the working purpose of the dog, and the desires of the owner.

The prevention of pet overpopulation, a serious and continuing problem in the United States, is one societal benefit of sterilization. Millions of dogs and cats are euthanized in this country each year. Spaying and neutering at an early age increases the chances that people who adopt animals will not relinquish them, Dr. Root-Kustritz said. Only 60% of people who adopt from a shelter comply with the requirement to have the animal spayed or neutered.

In surveys of people whose intact animals gave birth, 56% of canine litters and 68% of feline litters were unplanned. This is likely the result of ignorance of, or misinformation about, the animal's normal reproductive physiology, Dr. Root-Kustritz said.

She advised that dogs with no guardian should be spayed or neutered. If the owner is a responsible person who will not let the dog breed indiscriminately, however, she said she sees her role as a veterinarian to be one of education only.

Castration in male dogs decreases the incidence of testicular neoplasia, a tumor common among older animals. However, only 0.9% of dogs fall prey to this disease, which is easy to diagnose and cure. Castration also decreases the incidence of benign prostatic hypertrophy, which is seen in 75%–80% of dogs older than six years. Even here, morbidity is low, and castration is curative. A positive correlation has been noted between castration and increased lifespan; this may be because owners who pay for castration are typically more likely to take good care of their dogs and not allow them to engage in high-risk activity.

Despite these health advantages, sterilization can lead to problems. Multiple studies have shown a rise in the incidence of obesity in both male and female sterilized dogs. While obesity is the most common nutritional disorder in dogs, it can be controlled with proper diet.

Castrated dogs are two to four times more likely to develop prostatic adenocarcinoma, a high morbidity and high mortality glandular tumor. Only 0.2%–0.6% of dogs will develop this disease in their lifetime. Although most owners choose to euthanize a dog with this diagnosis,

Dr. Root-Kustritz cautioned against generalizing from these statistics. Prostatic adenocarcinoma is not hormone-dependent; therefore, there is no cause-and-effect relationship.

Osteosarcoma, or bone cancer, is a very aggressive tumor characterized by high morbidity and mortality. Castrated males are one to three times more likely to develop this disease, which occurs in 0.2% of the population—usually in older dogs. Large and giant breeds are most at risk, Dr. Root-Kustritz said. A study of Rottweilers showed a predisposition to the disease, so genetics may be as much of a causative factor as castration.

Also more common in large breeds is hemangiosarcoma, a tumor found in any organ with a large blood supply. The risk to castrated dogs is twice that of intact animals. Although it occurs in only 0.2% of all dogs, Dr. Root-Kustritz said surgery to remove the tumor is risky and mortality is high.

A higher risk of anterior cruciate ligament (ACL) injury, the most common orthopedic ailment in athletic dogs, was linked in one study to sterilization. Anecdotal evidence involving a relationship between a higher incidence of ACL injury and lower estrogen levels in women suggests that hormones play a role in the development of this disorder. Large breeds are more at risk, Dr. Root-Kustritz said. Billions are spent each year in this country on surgery to cure this ailment.

Benefits of sterilization in female dogs include a decrease in the incidence of mammary neoplasia, which occurs in 3.4% of all animals and is malignant in more than half these cases. Although the causation is not as clear as in humans, studies appear to show a hormonal basis for the disease.

“It has been demonstrated in numerous studies that you greatly decrease the incidence of mammary neoplasia in dogs by spaying them, and that you get the greatest benefit if they never go through heat at all,” Dr. Root-Kustritz said.

Pyometra, or uterine disease, is normally seen in older dogs that have experienced numerous heat cycles, when hormone levels are extremely high. Swedish studies show the incidence of this disorder is 15.2% by four years of age and 23%–24% by 10 years of age. Ovariohysterectomy (OHE) is curative; however, surgery is dangerous and post-OHE mortality is as high as 17%.

Problems associated with sterilization in females are the same as in males, including greater risk of obesity and development of some types of tumors, including osteosarcoma.

Spayed females also are at higher risk for transitional cell carcinoma, a tumor of the urinary tract. Although the incidence is only 1.0%, Dr. Root-Kustritz said mortality is high.

Environmental factors contribute to the development of this tumor and should be considered before making the decision to spay.

Urinary incontinence (UI) presents in 5%–20% of spayed females. Dogs with this disease tend to leak urine while they sleep. In addition to spaying, Dr. Root-Kustritz said risk factors include breed, and weight greater than 20 kilograms. Studies published in the Journal of the American Veterinary Medical Association (JAVMA) in 2002 and 2004 show a greater risk of UI in dogs spayed before three months of age.

Disorders in male dogs that benefit from castration generally have low morbidity and mortality rates, whereas the severity of negative consequences associated with the procedure appears high. While there are many reasons to sterilize a male dog, Dr. Root-Kustritz said the detriments appear to outweigh the benefits. She recommended assessing the need for castration on a case-by-case basis.

Similarly, while there are many benefits and detriments to spaying, the diseases that appear to be exacerbated by sterilization are fairly uncommon or have low mortality rates. Conversely, diseases that benefit from spaying are more common and have much higher morbidity and mortality rates.

Dr. Root-Kustritz said she recommends spaying after the age of three months to lessen the incidence of UI, but before the dog goes through its first heat. For most dogs, this means sterilization at about six months old.

An article she wrote on this subject, in which Dr. Root-Kustritz included information on risk factors by breed, will be published in the December 2007 issue of JAVMA.

Discussion

A participant asked whether there is research to back up the claim that spayed and castrated dogs show a higher incidence of aggression or fearfulness. Dr. Root-Kustritz cited a study done with the English Springer Spaniel that proved this statistically. However, she said, while spayed or castrated dogs might be predisposed toward aggression, this behavior can have multiple causes, and even intact dogs can become aggressive.

Another participant asked about the relationship between sterilization and premature epiphyseal closure. Dr. Root-Kustritz said studies with cats have shown spaying or neutering before the growth plates close can result in a slightly taller-than-average animal. Estrogen and testosterone are necessary for the growth plates to expand quickly during puberty, and then close. If sterilized before they go through that growth phase, the animal is never exposed to those hormones. She said there is no evidence that sterilized dogs are more susceptible to fracture.

Infectious Disease

Christine Petersen, DVM, Ph.D.
Iowa State University

Dr. Christine Petersen said she applauds the use of vaccines, but does not recommend their use for every disease.

While canine rabies is the primary strain that causes human deaths in the rest of the world, the disease has been eliminated in the United States, where cases generally originate in other species such as raccoons, bats, and foxes. However, 55,000 people died from rabies worldwide in 2006, 24 of them in the United States. To prevent rabies, Dr. Petersen said all dogs should be vaccinated, as well as at-risk humans such as veterinarians and animal shelter staff.

Intestinal parasites produce a condition that is generally very manageable in dogs. The eggs of hookworms or roundworms are spread to humans through dog feces. Dr. Petersen said a study of playgrounds in Connecticut found 30% of soil samples contaminated with roundworm eggs. These parasites also can be transmitted from bitch to puppy, so it is important that breeders follow Centers for Disease Control and Prevention guidelines.

Dr. Petersen noted that cases of brucellosis, a bacterial disease primarily found in the bowel, vaginal secretions, or semen, are on the rise in Iowa and Missouri. Occasionally puppies are exposed through mother's milk, but she said that if the bacteria are present in milk, the puppy probably acquired it *in utero*. The lack of reliable treatment leads many breeders to euthanize infected dogs.

"That's not much of a decision," she said, "as this disease isn't something you want in your kennel."

Brucellosis can cause disease in humans, so gloves should be worn when handling feces.

Though inexpensive screening tests are available, she said the tests have a high false-positive rate. The polymerase chain reaction (PCR) test, which tests for bacteria rather than immune response, allows infected animals to be identified sooner, potentially preventing exposure to other dogs. A positive culture confirms the presence of the disease while a negative culture means only that parasites did not grow in that particular specimen.

To prevent exposure to animals in a kennel, Dr. Petersen suggested that all dogs be screened with a rapid slide agglutination test, or tube agglutination test. Any positive results can be confirmed with an agar gel immunodiffusion test or culture. She recommended that retesting be conducted once a month for three months, and that new dogs be quarantined before breeding.

Since infected males can shed the bacteria in their semen for up to two years, Dr. Petersen suggested euthanizing culture-positive males. Treatment with antibiotics is only occasionally

successful, takes a long time, and does not usually result in a sterile cure. In exceptional circumstances bitches can be treated, but any future breeding should be done through in vitro fertilization, she said.

Tularemia, a tick-borne bacterial disease, is present throughout the United States, but is currently centered in Missouri. Four ticks are known to be carriers. Biting flies can also transmit the disease, especially in the Southwest United States. Symptoms of tularemia range from benign ulcers to lesions and glandular diseases in both dogs and humans. Although gentamycin has been used to treat the disease in humans, the efficacy of antimicrobial therapy for dogs has yet to be determined.

Leptospirosis is a potentially fatal disease that presents with kidney-, liver-, and vascular-related ailments. Herding dogs, hounds, and working dogs appear to be at greatest risk, Dr. Petersen said, although outbreaks have been reported after exposure to sources of standing water. Two studies showed 10–15% prevalence in the US Midwest. A study of veterinary clinics showed an incidence range of 4–33%.

The newest vaccines should be given twice a year and contain four strains, or serovars, believed to be tied to a decrease in seroprevalence. When clinical signs such as renal failure are present, Dr. Petersen said, an appropriate therapy is recommended. While antibiotics such as penicillin remain the treatment of choice, doxycycline appears to be the best route to eliminate the disease when it presents in the kidneys.

Canine influenza virus (CIV) is a highly infectious respiratory disease closely related to the virus that causes equine influenza. Although dogs are not traditionally carriers, an outbreak was reported among Greyhounds at a racetrack in Florida in 2004. Dr. Petersen said clinical signs of the disease include rapid onset fever, cough, increased respiratory rate, and bleeding from the nose. Nearly 100% of dogs exposed to the virus will develop the disease, although the mortality rate is only 5%, and is usually attributed to secondary infections.

The treatment for CIV is antibiotics, and Dr. Petersen said isolation from other dogs for seven days greatly reduces the risk to the rest of the kennel population. Since the virus is spread mainly by respiratory droplets, good hygiene practices are critical in preventing an outbreak.

Mosquito-borne West Nile virus (WNV) was introduced into the United States in 1999. It usually attacks birds such as crows and jays, horses, and humans, but Dr. Petersen said it is relatively rare in dogs. Symptoms include lethargy, poor appetite, frequent drinking, eye discharge, fever, and watery diarrhea. The disease then progresses to the next stage, where neurological symptoms like head tilt are more typically associated with classic WNV.

Leishmaniasis is a parasitic disease transmitted by the sand fly. It has been reported in the United States primarily among Foxhounds. Dogs typically present with the visceral form of the disease, which means the parasite has migrated to the vital organs.

Dr. Petersen said it is unclear how the disease spreads in the United States. A test was conducted on hundreds of sand flies, and none were infected. Humans who spend a great deal of time around infected dogs do not get sick. Anecdotal evidence suggests the disease may be transmitted through the blood, as Foxhounds developed it after a fight. It may be transmitted vertically, from bitch to pup, either through the placenta or in the mother's milk. If this were true, the pup's immune system would have difficulty recognizing the parasite as a parasite. This may explain why the antibody response in these dogs may take two to seven years to develop. The PCR test appears to be the most accurate means of diagnosis, although it can give false positive results.

Allopurinol is currently the main therapy for leishmaniasis; although there is no cure, treated dogs typically live two years after onset of the disease. Alternative experimental therapies are available as well.

The best way to protect against an infectious disease is to understand how it is spread and to take steps to prevent the spread among your animals, Dr. Petersen said. The proper use of disinfectant is important. In diseases spread through sexual contact or from bitch to pup, she advises against breeding.

Discussion

A participant asked about the availability of a national database of infectious disease outbreaks so breeders would know whether they should continue to vaccinate against all diseases. She said that because there has not been a positive test for brucellosis in Rhode Island for many years, she has stopped testing for it in her dogs. She asked whether that is advisable, as long as the dogs are not taken outside the region. Dr. Petersen said that although a national database would provide valuable information, to her knowledge none exists at this time.

The same attendee noted the need for education among veterinarians and physicians regarding zoonotic diseases—those that can be transmitted between animals and humans—especially in the wake of Hurricane Katrina and the anticipated pandemic flu. Dr. Petersen agreed, and said the American Veterinary Medical Association and American Public Health Association are taking a strong stand and encouraging more outreach.

Another participant said she would not stop testing her breeding dogs, though she has not seen a case of brucellosis in a long time. She suggested all breeders and veterinarians continue to immunize dogs, especially rescue dogs, from infectious diseases, regardless of the incidence in their area.

A participant asked whether any other breed had developed leishmaniasis. No others have come up positive, Dr. Petersen said, apart from a small number of Beagles who may have been exposed accidentally to an infected Foxhound.

Another participant asked whether dogs, like humans, could develop brucellosis from cows. Dr. Petersen said the bovine strain does not cross over into dogs.

Use of Probiotics: Benefits of a Balanced Microbiome in the Intestinal Tract

Gail Czarnecki-Maulden, Ph.D.
Nestlé Purina Research Center

Dr. Gail Czarnecki-Maulden said that 90% of all cells in the body are bacterial cells, with many residing in the intestinal tract, or gut. There are more than a billion bacteria in a gram of feces, and about half of fecal weight is made up of intestinal bacteria, or microflora. Beneficial varieties such as lactic acid bacteria coexist in the gut with potentially harmful bacteria. Dr. Czarnecki-Maulden defined microbiome as the state of equilibrium between the beneficial and harmful bacteria.

Potentially harmful bacteria in the system include those that produce pathogenic effects, and those that produce inflammatory enterotoxins, which may cause damage to the gastrointestinal (GI) tract. Enterotoxins can exacerbate arthritis in an affected dog, and decrease skin and coat quality, even though the animal does not present with diarrhea. These bacteria also increase the output of putrefactive substances that cause feces to smell. Many putrefactive substances are potentially harmful, Dr. Czarnecki-Maulden said; some have been linked to disease such as colon cancer.

However, beneficial forms of bacteria provide nutrients for intestinal cells by producing short-chain fatty acids, which are the main energy source for the intestine. These bacteria feed the dog's gut by consuming the food the dog eats. This process enhances nutrient absorption, Dr. Czarnecki-Maulden said. Short chain fatty acids have been linked to increases and improvements in mineral absorption. These bacteria also produce a significant amount of vitamins. For example, dogs get almost all of their Vitamin K from their gut microflora.

Dr. Czarnecki-Maulden said beneficial bacteria also promote a healthy immune system, keep the body primed and ready to fight infections, and block attachment of potential pathogens to the intestinal wall by producing toxins to kill the pathogens.

Animals that do not establish a normal microflora environment shortly after birth are much more susceptible to infection, and have poor immune function, more GI upset, poor reproduction, and a shorter lifespan.

Shifts in microflora balance can cause gut instability, resulting in diarrhea and a host of subclinical issues that may make the animal more susceptible to disease. Stress—whether good or bad—as well as travel, changes in environment, diet, or life style, poor nutrition, infection,

long-term broad spectrum antibiotic therapy, and aging can all upset the normal balance of intestinal microflora.

Probiotics, which are live beneficial bacteria such as lactobacilli, bifidobacteria, and enterococcus, can restore a healthy, balanced microflora environment. Dr. Czarnecki-Maulden said that to be effective, a probiotic must be able to survive in the GI tract, do its job safely, and remain stable until consumed.

Probiotics are highly strain-dependent. Researchers at Nestlé Purina Research Center in Switzerland studied 75 different strains of canine lactobacilli. Even though they were all very similar, she said, only 16 had potential probiotic activity. Each probiotic has specific antipathogenic effects, and target different diseases.

In humans, probiotics have been successful when used during or after antibiotic therapy, to prevent diarrhea in children, to treat travelers' diarrhea and inflammatory bowel disease, and, when taken by mothers prenatally, to decrease atopic dermatitis in infants. Unlike antibiotics, she said, probiotics do not produce immediate results, but they do alleviate symptoms over the course of a few days.

Numerous studies have proven the efficacy of the probiotic enterococcus faecium (SF68) in mice, humans, and dogs, Dr. Czarnecki-Maulden said. Researchers at the Nestlé Purina Research Center in Switzerland studied the ability of SF68 to improve the specific anti-giardia immune response in mice. The test group showed enhanced protection over the control group.

In another study, SF68 was tested on humans with diarrhea. After three days, 50% of those in the test group had no symptoms; after four days, 80% had no symptoms. Among the control group, 60% were symptomatic after four days.

From a clinical standpoint, Dr. Czarnecki-Maulden said, probiotics can be used to restore microflora balance in dogs with diarrhea, and to prevent or treat diarrhea associated with long-term antibiotic use. Success has been reported in cases where probiotics have been used with intermittent stress diarrhea and chronic diarrhea that is non-responsive to other therapies or which recurs after antibiotic removal. Probiotics also can be useful in non-clinical applications to help mitigate stress-induced diarrhea in sensitive animals, particularly prior to and during boarding or showing.

Dr. Czarnecki-Maulden cited data showing that bad bacteria tend to increase as a dog ages, while good bacteria decrease. Older dogs also tend to be more sensitive to dietary changes and other stressors.

Several studies conducted by Nestlé Purina have shown the benefits of probiotics, she said. In one, puppies fed a diet supplemented with SF68 had better fecal stability than those in the control group. Results showed that SF68 survives in the gastrointestinal tract, influences microflora balance, and enhances immune function and fecal quality in puppies.

Sources of probiotics include yogurt and supplements. However, not all products on the United States market contain the necessary probiotic cultures to work properly. Dr. Czarnecki-Maulden said a 2003 study of 19 commercial pet foods claiming to contain probiotics found that 75% had inaccurate labels, a number had one or more organisms missing, and several contained potentially pathogenic organisms. The study also found that canned dog food often remains on the store shelf too long for probiotics to remain alive. Microencapsulation can be used to help bacteria withstand the moisture and temperature fluctuations in canned food.

Dr. Czarnecki-Maulden said product labels should include:

- A guaranteed analysis of the number of live probiotic bacteria at the end of the product's shelf life
- A "best if used by" date
- A list of ingredients that shows the probiotic strain, confirms that it has FDA approval, and states that it has proven efficacy in dogs

Case Study: Hyperparathyroidism in the Keeshond

Richard Goldstein, DVM
Cornell University

Dr. Richard Goldstein said his case study was less about a particular disease of a particular breed than an example "of what it's possible to do today with a very involved breeding community, a health club, and researchers with today's technology." The experience of isolating the gene for primary hyperparathyroidism (PHPT) in the Keeshond points the way to "something that we can all be doing with all of our breeds."

The four parathyroid glands are situated on the thyroid itself, and the role of parathyroid hormone is to restore calcium levels when they drop. Calcium is also activated by Vitamin D, and 99% of it is stored in the skeleton.

Only six canine diseases lead to high calcium, and PHPT is the second most common, after malignant tumors. The relatively simple diagnosis makes the condition easier to identify than more complex diseases like hip dysplasia. However, the average onset at 11 years of age makes genetic testing more challenging than with diseases that appear in younger dogs.

A further challenge is that PHPT symptoms like weakness, trembling, and bladder or kidney stones may be missed, or attributed to a dog's age. The condition is almost always linked to a single, non-malignant tumor, and the resulting hormonal shifts can lead to kidney damage when unattended. With early diagnosis, PHPT is cured in 90% of cases.

PHPT tumors can be removed surgically, injected with ethanol, or treated with a needle heated at the tip. With any treatment, Dr. Goldstein said, aftercare is the biggest challenge. In the three to seven days following surgery, calcium levels drop sufficiently to show clinical signs of low calcium, and sometimes low enough to cause death. Treating the calcium deficiency is a simple matter if a veterinarian knows to look for it.

For some years, studies had shown that the Keeshond was over-represented among dogs with PHPT—one university calculated an odds ratio of 50:1 for a Keeshond to develop the condition compared to the average canine. After several “very prominent dogs in the Keeshond world” developed PHPT, individual breeders and the Keeshond Club of America approached Dr. Goldstein to study the condition. “When you look at a lot of success stories, there are always people in the breed who are very helpful in getting the samples and getting the studies done,” he said.

Establishing ties with the breed club was probably the most important step, Dr. Goldstein said, but it was followed by a sustained effort to collect pedigree data and determine the mode of inheritance. From there, the “biggest stepping stone” was to collect samples from healthy and affected dogs—a task that ran into definitional issues, since a healthy 10-year-old might still develop the late-onset disease at a later date. Researchers assembled a larger collection of samples to deal with possible errors in classification.

In the end, he said, the entire research project took only two years. With funding from a genetic research consortium at Cornell University, Dr. Goldstein’s team collected 180 samples, including 35 confirmed positives, and created a database of 1,647 dogs as a basis for designing pedigrees.

Although the pedigrees went back three to five generations, the late onset of the disease meant it would be difficult to get samples from live parents, and virtually impossible to get grandparents. However, from the available data, it became clear that the PHPT trait was autosomal dominant, not recessive, with partial, age-dependent penetrance. While the data showed a number of dogs listed as healthy, Dr. Goldstein said the easiest explanation was that they died of other causes before the onset of PHPT.

The other anomaly was that all the affected dogs showed one bad allele, but not two, leading Dr. Goldstein to conclude that the combination of two PHPT genes is lethal *in utero*.

Once PHPT was characterized as a dominant genetic disease, the team identified three candidate genes based on their links to comparable human syndromes. After all three of them were ruled out, the next step was to undertake a genome-wide scan, made possible by a two-year grant from CHF and sponsored by the Keeshond Club of America

Genome-wide scans can be based on linkage mapping within families, or association mapping across an entire population. While linkage mapping looks for shifts in relatively unstable

markers likely to change within a few generations, association mapping relies on more stable markers with greater longevity. The limited number of generations available for the Keeshond PHPT study dictated association mapping of a particular marker allele at the population level, which meant studying dogs as unrelated as possible.

The timing of the study was fortunate, Dr. Goldstein said, since it coincided with the introduction of a new SNP chip technology for genotype analysis. After the SNP system was adapted for canines by the Broad Institute at the Massachusetts Institute of Technology, the research team used it to test genetic differences between 27 affected and 42 unaffected animals.

Somewhat to the surprise of the research team, the analysis revealed one chromosome with a statistically significant difference between affected and unaffected subjects, where most or all of the markers were shared by the affected dogs but different for the unaffected ones. The relevant chromosome contained three candidate genes, two of which were easily ruled out by sequencing.

When the researchers studied the remaining gene more closely, they found that the relevant mutation occurred in the promoter region. The result was that tissue RNA was normal, but the quantity of RNA was a problem—and upon further analysis, Dr. Goldstein said, the mutation showed up in every affected dog in the study.

The mutation was also present in some of the unaffected animals, indicating that they were likely to develop PHPT later in life. Dr. Goldstein said that kind of test result can be difficult for a breeder, but he recommended careful monitoring rather than drastic action: the disease may never appear, and if it does, it is treatable.

At the end of the two-year process, the research team had developed a genetic test for PHPT and made it available to the Keeshond community. Dr. Goldstein said the key success factors were “the ability to get samples from a variety of owners and not just from Ithaca, New York; the ability to get seed funding from CHF for a study of dogs for dogs; and the availability of the technology to run the study. It all came together at the perfect time.”

Discussion

A participant asked whether the PHPT mutation is present in younger dogs. Dr. Goldstein said tests of affected 10-year-old dogs had determined that their blood and muscle tissue were heterozygous, but that their PHPT tumors had shown only affected copies. Similarly, when surgeons remove tiny samples of parathyroid gland and surrounding fat cells, they find normal and affected tissue a millimeter apart. The same phenomenon is quite common in human endocrine cancers. The theory is that an individual might be carrying the gene, but cancer develops when the gland receives two defective copies of the chromosome.

Canine Health Information Center (CHIC)

Eddie Dziuk
Chief Operating Officer
Orthopedic Foundation for Animals (OFA)

Since its official launch at the 2001 CHF conference, CHIC has had valuable news to report back to the Foundation, Eddie Dziuk told participants.

In 2003, CHIC presented a detailed overview of its purpose and potential. The 2005 conference marked the launch of the Center's DNA repository. This year, Dziuk gave the audience a tour of the new online health survey introduced by the OFA.

Canine health databases are a tool to help breeders make more informed breeding decisions, produce healthier dogs, and reduce the incidence of genetic disease. The concept for CHIC originated within the AKC delegate body, and CHF and OFA have sponsored the Center jointly since its inception. Today, CHIC is working with nearly 100 Parent Clubs, representing more than half of the AKC-recognized breeds, and more than 40,000 dogs have been tested. Test results are posted online in a publicly accessible database.

Dziuk emphasized that the test protocols for specific breeds are set by Parent Clubs, not by the CHF or the OFA. "This is the Parent Club driving the process," he said. "This is the Parent Club's recommendation on what the appropriate screening should be for the particular breed."

The purpose of the system is to encourage health testing and awareness, not to define normalcy; a CHIC number does not imply that a dog is free of defective genes. Breeders can do a better job of avoiding undesired traits if they know where to find them in a breed population. CHIC supports that effort by providing a consolidated, central database, with appropriate infrastructure and staff, at no cost to the clubs.

The DNA repository was established to speed the research process by eliminating the 12- to 24-month search for samples that had previously been a necessary first step for canine genetics researchers. The repository offers the research community a standing bank of samples from healthy and diseased dogs, with the optimized family groupings often required for research purposes. The system allows breeders to take advantage of future DNA-based tests as they become available, and fosters a team environment between breeders and researchers that improves the likelihood of genetic discovery.

"It's all working together toward a common goal and finding the genetic marker for a disease," Dziuk said. "Independently, it probably would never happen."

Early on, the repository had to balance the pros and cons of blood versus cheek swab samples. Blood yields more DNA of higher quality and can be used with all scientific applications, including emerging technologies like single nucleotide polymorphism (SNP). However, blood

tests are more invasive, cost more, and cannot be done by a breeder working from home. While swabs yield less DNA of poorer quality, and cannot yet be used with new technology, they cost less, they are non-invasive, and their simplicity drives a higher participation rate.

After considerable discussion, CHIC decided to offer a dual solution and leave it to owners to choose their sampling method. "Blood is much preferred," Dziuk said. However, if it is a choice between representing a dog and not including it at all, "we're certainly interested in having a swab."

The repository was launched two years ago with a "wildly successful program" to collect samples, pedigrees, and health histories for nearly 1,000 dogs. Working with the Golden Retriever Club of America, Dziuk said CHIC collected "a wonderful mix of healthy dogs, diseased dogs, young dogs and old," providing the diversity a researcher requires to launch a health study. Two years later, the repository has samples representing more than 80 breeds, and increased its total sample count from 3,000 in August to 4,000 in October after holding DNA collection clinics at about a dozen breed events.

Although CHIC still considers the repository to be in its infancy, it already has success stories to report. When Drs. Cheryl London and Kerstin Lindblad-Toh needed suitable samples for a study of mast cell tumors in Golden Retrievers, CHIC was able to provide samples, pathology reports, and pedigrees for diseased dogs, as well as a number of animals for the control group.

The benefit to the breeding community was brought home in mid-October 2007, when a breeder approached CHIC for genetic information on a sire that had recently died. The breeder was looking for evidence of Progressive Retinal Atrophy (PRA), and requested a portion of a past sample to test the dog's status.

"What a wonderful tool for this particular breeder," Dziuk said. Rather than having all the offspring tested, the breeder simply went back to the deceased dog's DNA.

Dziuk noted that the DNA repository had been the vision of the late Bob Kelly. He acknowledged Kelly as a close mentor and a good friend and credited him with "the foresight to see this through."

The OFA online survey was introduced in September with two pilot breeds, the Labrador Retriever and the Australian Cattle Dog. Dziuk said each breed survey is distinct—while the Labrador Retriever Club of America asked questions at a 60,000-foot level, the Australian Cattle Dog questionnaire drills down to specific health concerns within the breeder community.

The Labrador Retriever survey received more than 600 responses between Labor Day and late October, while the Australian Cattle Dog site generated more than 275 in its first few weeks of operation.

In the years ahead, Dziuk said he hopes to see a full listing of breed-specific health surveys on the OFA website. The surveys would each be developed by the individual Parent Clubs, enabling owners to answer the most pertinent questions for their breeds.

The centralized approach would give clubs access to technology and technical expertise that might otherwise be beyond their reach, and would eliminate concerns about sharing confidential test results within a group of breeders. Dziuk urged participants to get in touch if they want move quickly in the direction of an online survey. CHIC is a small organization, he said, and the limited resources available for online surveys will be devoted to the breeds that step up first.

Discussion

An OFA staff member said she is tremendously excited about the online survey, noting that her Parent Club's in-house survey had only collected 61 responses for a breed that registers a very large number of dogs per year.

A participant asked whether veterinary clinics had been invited to place patients' blood samples in the DNA repository once definitive diagnosis had been made. Dziuk said CHIC has had limited staff time for outreach and education, and has concentrated its efforts on major teaching hospitals and veterinary schools.

A representative of the Australian Cattle Dog Club of America described the online survey as "an enormous service," particularly for smaller breed clubs. "With good outreach and communications, it is very possible for even the small clubs to get a lot of interested folks" to fill out the survey, she said. "It's almost contagious once it gets going."

A veterinary pathologist urged other participants to allow autopsies after their dogs die, noting that "a tremendous amount of information" would otherwise be lost.

A participant asked for clarification of the different DNA banks in which clubs are invited to participate. Dziuk said the CHIC repository is completely separate from the AKC donor program, which uses a single swab for a 14-marker panel that verifies parentage and provides for studbook integrity. The rules governing the AKC's DNA bank prohibit other uses for the data, including health research—and, in any case, the quantity of DNA is insufficient for research purposes.

The CHIC repository is an alternative to the "sample fatigue" many breeders are experiencing, he said, since it is available to all the researchers who have been requesting DNA for individual studies. Most of the researchers "are wonderful about collaborating" but, before the repository, nobody kept track of the samples available in different labs. "There was no centralized database to report the existence of that sample, and that prevented the collaborative spirit from moving forward."

A participant asked whether her club could arrange CHIC numbers for dogs previously sampled by a university laboratory. Dziuk said arrangements could probably be made, though CHIC would probably go back to owners to update the dogs' health data and gather additional detail.

A participant pointed out that DNA could be extracted from any tissue and suggested that even material from dewclaws could be added to the database. Dziuk agreed that any cell nucleus will be rich in DNA, but explained that the two laboratories associated with the CHIC repository are optimized for blood samples and cheek swabs.

Day Two: Welcome

A. Duane Butherus, Ph.D.
Chair, Grants Committee
CHF

Steve Remspecher
Director of Marketing
Nestlé Purina PetCare

Dr. Duane Butherus said breed health liaisons play a crucial role in helping the Canine Health Foundation fulfill its mission to raise money efficiently, manage it well, and spend it wisely.

"We need you from the standpoint of telling us what the problems are in your breed. There's no way we can look into those problems if we don't know what they are," he said. "The better, more accurate and up-to-date your health surveys are, the better we can serve you by finding the best researchers to work on these problems."

Dr. Butherus explained that CHF issues an annual request for proposals to over 5,000 researchers around the world. The document lists the top diseases identified in breed health surveys, as well as conditions of special interest to specific breeds.

Grants are divided into two categories. Projects ranging from \$12,000 to \$250,000 in value are assessed annually, and are subject to peer review and numerical ranking before they are forwarded to the Grants Committee. This category of work receives about \$1.8 million in annual funding.

A series of smaller grants, called ACORNs, worth no more than \$12,000 plus 8% overhead, was initiated two years ago at the suggestion of the late Bob Kelly. Grants are reviewed weekly, and decisions are made within 30 days of receipt. More than 100 grants have been approved, and the program has been particularly successful through the revolution in genomics. "Things that

used to cost us \$100,000 over two years can now be done for \$12,000 in one year," Dr. Butherus said.

He identified prevention, treatment, and cure as the Foundation's three-pronged approach to funding. Genomics research comes under the heading of prevention, which received 78% of the major grant money approved in January 2007. The basic operating principle, he said, is that "you don't eradicate good dogs from your breeding programs because they're carriers. You substitute for them so you don't shrink your gene pool. But that means knowing who is and who isn't a carrier."

Treatment of chronic canine diseases received 13% of the grant fund, with the balance directed to methods of reversing the effects of disease, mainly by applying stem cells to cardiac problems. "We're excited about this," Dr. Butherus said. "We're not going to put all of our funds in that pot, but we're going to start and see what kind of results we get."

CHF's key priority is to get information back to Parent Clubs, through breeder symposia and the biennial conference, he said.

Steve Remspecher noted the large number of first-time attendees at the conference. "If we've done our job," he said, "every one of them will go back and be an ambassador for the Canine Health Foundation and get the message out."

Nestlé Purina's support goes back to the first Parent Club health conference in 1997. He described other ways in which the company helps CHF to deliver its message. That commitment has endured "because it's the right thing to do. As a world leader in pet nutrition, Nestlé feels it must take a leadership position in finding cures for diseases in dogs around the world." With more than \$20 million invested in canine health research, "we're making that happen." And "for this, we owe each of you a big thank you. It's because of you that this organization is in place and is working."

Remspecher urged each Parent Club to increase its funding for health research. Clubs currently hold about \$3 million in donor-advised funds and "there's an opportunity to spend that money," possibly in coordination with other clubs that face similar health issues.

He challenged participants "to overcome the obstacles that have inhibited your club's ability to address the health issues facing your breed, whether those obstacles be financial, the lack of a plan, club politics, or that they're just not on the radar screen. We're here to help."

Keynote Address: Cytotherapeutics in Veterinary Medicine

Rick Vulliet, DVM, Ph.D.
School of Veterinary Medicine,
University of California-Davis

Dr. Rick Vulliet said the history of therapeutic drug development for humans hit a wall around the turn of the century with the inability to cure diseases associated with aging, chronic degenerative disease, and diseases associated with cell loss.

Today, stem cell research holds out the prospect of curing conditions like cardiovascular disease, autoimmune deficiencies, diabetes, osteoporosis, and cancer. Each of these diseases takes a major toll on human populations, Dr. Vulliet said, and many of them have canine equivalents.

Encouraging as it would be to claim that cell loss diseases could be cured by stem cells, however, “the reality is still pretty foggy,” he said. “We have cured thousands and thousands of mutant mice, we’ve made progress in laboratory rodents, but the question is whether we’re ready to move into naturally occurring diseases in real patients, real dogs with real diseases.” While he said he believes they are, he said there is still no guarantee that the techniques will work.

Dr. Vulliet said the first stem cell with the ability to grow all the tissues of the body was a fertilized rat zygote, making *in vitro* fertilization one in a series of treatments that eventually migrated over from veterinary medicine to human health. The first successful human stem cell study demonstrated that muscle, nerve, pancreatic, and other cells could be grown in culture under special conditions.

From that point, ethical concerns began to proliferate, amid concerns about applications that bordered on science fiction. “No serious stem cell researcher supports reproductive cloning, and yet that’s where most of the ethical stuff comes from,” Dr. Vulliet said. When one private company grew a human clone to the stage of about six cells, the work “was soundly denounced by the scientific community.” By contrast, strong support exists for therapeutic cloning of specific cells for specific purposes.

Unfortunately, the majority of attention and resources has been devoted to the ethical debate, rather than to actual stem cell research. “Our job is to work out what we can do,” Dr. Vulliet said. “Then we’ll figure out whether we should do it or not.”

Beyond fertilized zygotes and embryonic tissues, there are several sources of cells that can change from one tissue type, or phenotype, to another. Dr. Vulliet said his work has focused on canine, equine, feline, and rodent bone marrow. “They all seem to grow very well in the same

conditions, with similar morphology and response." The cells can grow into bone, chondrocytes, myocytes, tendon, ligament, and possibly nerve cells, a degree of versatility that makes stem cells a promising research focus for many breeds.

In 2001, a research team at the US National Institutes of Health (NIH) induced the equivalent of a myocardial infarction in rats, injected stem cells, and found that the cells grew in the infarcted tissue. The study could not be replicated, but researchers in Germany are still conducting follow-up clinical trials. The results have suggested a possible direction for research on heart disease in Doberman Pinschers.

Dr. Vulliet's specific research target was dilated cardiomyopathy (DCM), a heart condition common among Dobermans. In DCM, the heart no longer contracts the way it should, and loses the ability to pump blood. Humans with the same disease are placed on the transplant list, where 16,000 are currently waiting for procedures that take place at a rate of 4,000 per year.

"No one talks about the other 12,000, but I suspect we know what happens to them," Dr. Vulliet said. The NIH should be concerned, he said, "and they should be funding Doberman studies. We're working on it."

An initial series of safety studies revealed a variety of problems with myocardial stem cell treatment, including arrhythmias, micro-infarctions, and clogged arteries where the cells were introduced. Dr. Vulliet said growing techniques, handling methods, and routes of administration have all been revamped in the hope of developing a protocol that can be used on an animal patient.

Unexpectedly, the trans-differentiation the stem cells exhibited *in vitro* did not occur in the initial trials. Benefits may have resulted from improved blood flow through the heart, activation of the immune system, donations of mitochondria, or cell fusions, but "the bottom line is that we don't know how the cells work at this point. It's a very active area of research, not only in dogs, but in humans" and at this point mostly in rodents.

One recent paper reported a statistically significant improvement in rodent heart function following the introduction of stem cells from human bone marrow. The benefit persisted after the cells disappeared, however, suggesting that the cells themselves were not the source of the benefit.

In another NIH study, researchers investigated the effects of adult bone marrow stem cells on spinal cord injury in rats by dropping a precisely calibrated weight on the rat's exposed spinal cord. Seven days later, they added adult stem cells to the injury site and found significant improvement in recovery of the spinal cord to the injury. This improvement was supported by histological techniques as well as functional improvements. The rats showed improved function, likely because the stem cells stabilized existing cells and helped more of them survive the injury. Dr. Vulliet said a similar approach might work with degenerative myelopathy (DM)

in German Shepherds, although “you’ve got some logic leaps” in taking a treatment for acute injury in a rat and applying it to chronic injury in a dog. To owners who volunteer their dogs for a study, the message will be that “you’re participating in research. We don’t know if these things work. We really want to find out.”

The rodent spinal cord study has been replicated successfully at several laboratories, and Dr. Vulliet said this suggests it would be appropriate to attempt similar studies on dogs with DM. Some veterinarians have objected that dog studies are premature without better data, “but I also know what’s going to happen if we do nothing. That’s your choice as owners.”

Researchers have also injected stem cells into the tracheas of rodents with idiopathic pulmonary fibrosis (IPF) to reduce collagen deposition and scarring of lung tissue following treatments with bleomycin, an antibiotic and anti-cancer drug. Here again, the evidence is limited to laboratory tests on rodents, but “if you have an animal with IPF and you go to your vet, we can only give you symptomatic relief. There’s nothing to treat the disease.” The disease causes 40,000 human deaths per year in America, and the only treatment option is a lung transplant.

Dr. Vulliet also presented results on stem cell treatments for lipoprotein lipase (LPL) deficiency, a genetic mutation that appears in Miniature Schnauzers and Beagles, in felines, and in one in a million humans. The condition defines about 80 different mutations that all occur in the same enzyme.

In diagnostic tests, the blood serum drawn from an LPL-deficient is milky white, when it would normally be straw-colored. Two days after treatment with about 50 million bone marrow stem cells, the opacity of the blood serum declined for about four or five days. After a second treatment, it dropped farther and stayed down for longer. The cells were administered through a standard injection to the jugular vein and involved very little pain to the animals. “In the global scheme of things, compared to dying, I think it was relatively minor,” he said.

Lung tests showed an increase in lipase activity after the first treatment, and a larger increase after the second dose. Most astonishing of all was the behavior change in a cat that Dr. Vulliet described as “the second-dumbest I’d ever worked with.” Before treatment, the cat “just wanted to sit in your lap and shed,” with no behavior and no curiosity. After receiving 100 million stem cells, she began grooming herself, gained some weight, and showed far more complex behavior.

Already, Dr. Vulliet said, the discovery of plastic cells is rewriting pathology textbooks. Until now, physicians thought the week after an injury was characterized by inflammation, cell death, and an influx of inflammatory cells. The new theory is that an influx of regenerative cells begins after a week or two, and is followed by repair and scar formation. In some canine diseases, such as IPF in West Highland White Terriers, there is likely an exaggerated inflammatory response, followed by decreased regenerative cells and more scarring. The condition might now be addressed by infusing regenerative cells to reduce scar formation. “This is in contrast to what I was taught in pathology 25 or 30 years ago, but we really don’t know how this will play out.”

With limited information available, “anyone who donates their dog to this study is a hero,” Dr. Vulliet said. The other option is to wait 15 years for a standard human drug to be approved, and then look for canine applications. “With a real dog with a real disease right now, what are you going to do?”

He noted a study of humans with Parkinson’s disease in which the test subjects showed excellent results after a year, but fell behind the control group by the end of the second year. The results led the NIH and the US Food & Drug Administration to declare a moratorium on future human stem cell studies, leaving canine and other animal studies as an option the NIH should support.

Discussion

Dr. Butherus said CHF hopes to set up three regional stem cell therapy centers serving most major communities in the continental United States, where owners can bring their dogs to have bone marrow extracted and stem cells implanted. The overall approach reflects the Foundation’s three-pronged commitment to prevention, treatment, and cure. Dr. Vulliet said the regional approach was designed to minimize travel distances for the animals. “Many of you think shipping dogs is no big deal,” he said, but “it’s hard enough with a healthy dog, much less with a sick dog.”

Citing Albert Einstein’s observation that “imagination is better than knowledge,” a participant urged CHF to fund creative approaches. “For the money lenders, for research, please look at people who have imagination, not at the status quo of what’s accepted in science,” he said. “There will be no breakthroughs with that.”

A participant suggested that a continuous procedure might be needed to extend the temporary improvements in the Parkinson’s study.

Another participant said any request to owners to ship their dogs would “run smack into the human-animal bond. I might bring you my dog, but I’m not willing to let you have my dog.” Dr. Vulliet said it might be possible some day to have local veterinarians ship bone marrow by courier for extraction, then send stem cells back for implantation. “I can’t do that right now because I really don’t know what I’m doing. I couldn’t afford to send you a batch of cells, have them die in transit, and get a false negative.”

In response to another series of questions, he said stem cell implantation rarely leads to immune reactions.

Cardiology and Stem Cells

Mark Oyama, DVM, DACVIM
University of Pennsylvania

Cardiac conditions like mitral valve disease, dilated cardiomyopathy (DCM), and congenital heart disease are widely prevalent in dogs, Dr. Mark Oyama said. However, diagnosis is fairly expensive, and treatments are generally limited to relieving symptoms rather than addressing the root causes of disease. For most acquired heart disease, the best veterinarians can do is try to alleviate symptoms and maintain quality of life.

Mitral valve disease and DCM are particularly frustrating for the breeding community, since they appear in the middle to later years of life, after dogs are past breeding age. Both can probably be traced back to a genetic cause, though nutritional, toxic, and infectious components cannot be ruled out.

“The majority of breeds have some liability for coronary disease,” and there have been advances in identifying heritability patterns and possible causative genes, he said. “But as we approach a day when we understand the gene or genes that cause these diseases, we’re looking at additional therapies, and that’s where stem cell therapy comes into play.”

Dr. Oyama described DCM as a “global weakening” of heart muscle cells, leading to poor contractility of the heart muscle and progressive dilation of the heart. The progressive loss of function leads to buildup of fluid, exercise intolerance, abdominal distension, and poor appetite. The final outcome of the disease varies by breed: while Boxers are more likely to collapse from arrhythmic sudden death, most Great Danes succumb to heart failure. Despite best efforts, the one-year survival rate after a diagnosis of DCM is 15% across affected breeds, and about 50% in humans.

Until very recently, researchers believed that certain adult organs like the heart and brain were “terminally differentiated,” so that cells could not be replaced once they died. More recently, studies have revealed populations of adult stem cells in the bone marrow, liver, and heart that might rebuild these tissues. Dr. Oyama cautioned that “the intensity of that response is relatively low,” and not robust enough to repair major damage. However, the existence of stem cells in heart muscle tissues is now well documented.

This suggests two possible avenues for stem cell therapy: harvesting cells from other parts of the body, or augmenting native populations in the heart. Cells from bone and skeletal muscles have very good regenerative properties, and bone marrow cells can differentiate into nervous tissue, bone, or heart muscle. “They’re really quite plastic, or flexible, in what they grow up to be,” he said. “It’s all about their environment, nature versus nurture.” Initial human trials have shown improved contractility, although the study groups of 10 or 20 were far short of the thousands needed before a new drug can be approved.

So far, most successful human or animal trials have had to do with myocardial infarctions, Dr. Oyama said. In contrast to a chronic genetic condition in which all the heart cells eventually degenerate and die, the studies focused on a single event of poor blood flow in one part of the organ. Use of autologous stem cells sidesteps issues related to cell supply, transplant rejection, and broader ethics, and there is hope that skeletal cells will develop very similarly to heart muscle if they are placed in the right growth environment.

After extracting the cells and labeling them with fluorescent dye, the next step is to return them to the damaged heart in the least invasive manner possible, while covering the widest possible area of the organ. One challenge is that stem cell solutions contain a variety of different cell types, including fibroblasts that would grow into scar tissue. Some papers have suggested that up to 50% of injected stem cells are “garbage cells,” Dr. Oyama said, but researchers are still learning how to purify the solutions.

Once the cells are ready for implantation, the least invasive delivery method is a catheter from the femoral artery into the left ventricle of the heart. Stem cells are relatively large compared to the capillaries at the end of the coronary artery, which are only one cell thick. Researchers believe that the stem cells sometimes wiggle through to the surrounding heart muscle, into an environment “that will hopefully induce them to differentiate into functioning, working muscle tissue.”

Dr. Oyama said his laboratory injects between one and five million cells per treatment, since a larger volume will block the smaller coronary arteries. The most successful trials so far have involved up to three million cells, which is in itself a limitation, since the total number of injected cells is minuscule compared to the billions that make up the heart.

However, tests show that the cells do stay in the heart, rather than dying off or being flushed through the system, and the benefits in human trials seem disproportionate to the number of cells injected. Researchers are now considering that the treatment might benefit patients by drawing native stem cells to the damaged area, or by secreting some beneficial hormone that has yet to be identified. Follow-up studies have shown that granulocyte colony stimulating factor (G-CSF), a protein, produces considerable cell proliferation, Dr. Oyama said. It appears to mobilize native populations of stem cells, though the cells seem to have less ability to find the area where they are needed.

Beyond cytotherapeutics, Dr. Oyama identified gene transfer as an opportunity to correct primary and secondary deficiencies, rather than trying to cure them. Once a target has been identified, he said it might be possible to “infect” it with a virus that contains an active, healthy copy of the defective gene. The technique has been used to arrest mucopolysaccharidosis (MPS) in dogs, and it may be a useful treatment for muscular dystrophy in human children and Golden Retrievers, and in a rapidly progressing form of DCM associated with Portuguese

Water Dogs. "Here, the emphasis isn't so much on replacing the damaged cell. It's on replacing the gene in the cell that makes it damaged."

Discussion

Dr. Butherus asked whether new heart tissue grown from stem cells integrates automatically with the existing muscle. Dr. Oyama acknowledged that heart cells must act in close coordination to ensure a smooth, steady heart rhythm, but that myoblasts taken from skeletal muscle "aren't very good at making little plug-ins with their neighbors." As a result, human health researchers have concentrated their efforts on bone marrow stem cells.

A participant asked whether there are any differences between late-onset heart disease and the mitral valve disease that often occurs earlier in Cavalier King Charles Spaniels. "Unfortunately, we don't really know," Dr. Oyama said, but the evidence points to two different conditions.

A participant asked whether it would make sense to inject cells into Dobermans during the occult stage of the disease, before the heart fully dilates. Dr. Oyama said that would be unwise until more is known about the long-term safety, efficacy, and survival of stem cells. He agreed that it would be worthwhile to harvest bone marrow cells while a dog is still healthy, rather than compromising a sick animal with sedation or anesthesia, though it is still not known whether these cells can be frozen over long periods of time.

Cancer Stem Cells: A New Way to Look at an Old Disease

Jaime Modiano, DVM, Ph.D.
University of Minnesota

Dr. Modiano called cancer stem cells the "evil twin sister" of therapeutic stem cells, but said the similarity is no reason to doubt or fear the possibilities of regenerative therapy using stem cells.

Noting that dogs and humans have a common concern with cancer, he asked for a show of hands from those who had not been affected in some way by cancer, either human or canine. Only a few participants raised their hands.

Cancer is a conglomerate of conditions that represents a huge public health problem, Dr. Modiano said. It is the primary cause of death for adults under age 85, and the secondary cause for adults over 85 and for children.

He described cancer as the uncontrolled proliferation of cell growth, starting with a single cell. Playing a short video clip of a tumor's progression, he said that cancer cells continue to divide at the expense of normal cells around them. They grow, and then recruit blood vessels, and in

the final stage, called metastasis, the tumor cells invade the bloodstream and travel along vessels to infect other tissues.

Dr. Modiano said that while he has been told by many lay people that cancer is caused by walking under power lines, using a cell phone, being zapped by cosmic rays, or eating commercial dog food, the actual cause is life. Being alive means an inherent risk for cancer with every cell division, he said, and life itself is the biggest risk factor for cancer. He gave what he called the Breen/Modiano Axiom: "Cancer is an inevitable consequence of mammalian evolution."

Because nature has limited tools to work with, Dr. Modiano said, cells might try to revert to an earlier state. That may be where cancer starts. A long life exponentially increases the risk of cancer.

The lifetime risk for cancer in both dogs and people is between 30% and 50%, and cancer represents the leading cause of death for 50% of dogs aged seven and up. For some breeds, it is also the most common cause among diseases leading to death. For example, he said, one study showed that 60% of Golden Retriever deaths are from cancer.

While most cancer is treatable, the majority of cancers are not preventable with current strategies. It is possible to reduce the risk of some cancers—for example by using sunscreen, and quitting, or never starting, smoking. Some studies show that lean, fit animals have a reduced incidence and fewer problems with cancer, suggesting that a good diet and regular exercise also help.

"For humans, it's hard to get a handle on genetic factors," Dr. Modiano said. "That's where dogs come in." Statistics show that certain breeds, such as Boxers, Golden Retrievers, Labrador Retrievers, Basset Hounds, Doberman Pinschers, and German Shepherds, are at higher relative risk of lymphoma. Others, including mixed breeds, Pomeranians, Poodles and Chihuahuas, are at a lower risk. Dr. Modiano said researchers have speculated that small breeds may have less risk because they require fewer cell divisions to grow their smaller frames, but more research is needed. Recent research into insulin-like growth factors suggests that the risk of cancer in dogs may increase with body mass, but this hypothesis has not been proven.

One study that looked at 1,262 dogs of various breeds found that the average age for all dogs developing lymphoma was 9.5 years. Some breeds had a lower average age, including Golden Retrievers at 8.5 years and Rottweilers at 6.8 years. These numbers represent statistically significant deviations from the average, Dr. Modiano said, and could be evidence of less genetic diversity within a given breed. He noted that the study suggests a risk resulting from whatever makes up a breed's genetics. A study of 100 dogs with osteosarcoma showed similar numbers and age distributions. However, Dr. Modiano said, the meaning of these differences is still unknown.

Another osteosarcoma study tracked the survival of dogs with the disease over time according to the type of treatment received. Dogs receiving no treatment survived for less than one month; those receiving palliative treatment did not fare much better. Dogs with amputations survived longer, and those receiving the current standard of care had the best results of the four options studied. Dr. Modiano said researchers have found some genetic markers that seem to predict which dogs will do well and which will not.

Cancer cells are characterized by a series of mutations, self-sufficiency, reduced sensitivity to anti-growth signals, limitless replication, and the ability to evade apoptosis, programmed cell death or "suicide." A study at Johns Hopkins that sequenced 22 human tumors found that on average, each cancer cell contained 90 mutations, Dr. Modiano said.

Although many urban myths circulate regarding cancer, no single cause can be pinpointed. Toxic compounds can increase susceptibility, but even so, they are not a sure predictor. For example, Dr. Modiano said, only 20% of smokers get cancer. "There are many risk factors, but they only come together to produce cancer in one of five in this group."

While the existence of cancer stem cells is supported by compelling information, Dr. Modiano said, how they are formed is still unknown. If cancer stem cells really are like stem cells, they should be able to self-renew and give rise to heterogeneous reproduction. Studies of human chronic myelogenous leukemia done in Denmark and in Texas have shown that cancer stem cells do share these properties.

Dr. Modiano noted that this has implications for the conventional model for resistance and relapse, in which cancer returns after treatments kills the cancer cells. One hypothesis is that if treatments introduce mutations in cancer stem cells, they may become resistant to therapy, return, and then metastasize. If this is the case, Dr. Modiano said, researchers should try to develop treatments that specifically target cancer stem cells.

One small study of nine dogs showed that the presence of more stem cells was linked to a shorter survival time, suggesting that the presence of these cells may be predictive.

Is Canine Medicine Ready for Stem Cell Therapy (Cytotherapeutics)?

Moderator:

Dr. Butherus
Chair, Grants Committee
CHF

Panelists:

Dr. Jaime Modiano
Dr. Mark Oyama
Dr. Rick Vulliet

A participant thanked the panelists for “giving us hope” and asked about the correlation between levels of insulin-like growth factor (IGF) with cancer.

“If the answers were easy, a lot of smart people who have been working on the problem would have already found them,” Dr. Modiano said. IGF is not simplistic: a big dog with IGF does not automatically get cancer, and IGF may have nothing to do with cancer. One study of IGF inhibitors showed that even though the drug worked biologically, it did not work therapeutically, and side effects were unacceptable. This question presents an opportunity for further research.

Another participant asked the panel to address information suggesting that rates of osteosarcoma are high in animals that have been sterilized.

Dr. Modiano said that one 2003 study based on a survey of Rottweiler owners found that dogs neutered before the age of six months seemed to have a significant increase in bone cancer later in life. However, other researchers have tried without success to reproduce the data in clinical and university studies. Dr. Modiano recommended that pet owners consider all the reasons their dog should or should not be spayed or neutered, and base their decision on that rather than on fear of cancer.

A participant asked about genetic markers for osteosarcoma and the efficacy of so-called “organic” or “natural” treatments.

Most drugs are derived or modified from plant compounds, and doctors have exploited some of those poisons to kill cancer cells, Dr. Modiano said. However, to kill cancer, most of these compounds also kill some healthy cells. Noting, “Socrates died from drinking an all-natural herbal solution,” he said dog owners must be careful of “snake oil” and use caution when assessing products that are promoted using the terms “organic” or “natural.”

Dr. Modiano said researchers are looking for genetic markers for bone marrow cancers, but have yet to prove that certain markers are more associated than others with those cancers.

A participant asked about spontaneous remissions, noting that while they are rare they do occur, and enquired about the future of cancer vaccines.

Dr. Vulliet said he feels researchers will solve the mysteries of stem cells and cancer at the same time. "When we know why stem cells differentiate, we'll know why cancer doesn't." The body's natural immune system monitors the body and kills problematic cells before they can become cancerous; for this reason, Dr. Vulliet said, cancer could be described as a failure of the body's surveillance system. Stem cells could promote better immune responses and help the body to renew itself.

Another participant asked the panelists how they decide where to focus their research, and how individuals and clubs can determine where to direct their contributions.

Dr. Oyama said that each technology builds on the others, working in different but complementary ways, and suggested that donors consider researchers' study information to see which are the nearest to paying off. Dr. Vulliet said that two of every 10 grants he reads should not be funded, and at least five seem worth funding; however, usually only one or two can be funded.

A participant said that several dogs in one line in her breed club have come down with DCM, and that "one stud is obviously a carrier." Dr. Modiano suggested that she talk to Dr. Jerold Bell regarding breeding strategies for dogs with heritable diseases.

Dr. Vulliet noted that the current allocation of research funds is heavily weighted towards prevention, with much smaller percentages allocated to cures and palliative care. "We don't know [whether the balance is right], but we're thinking and would like your input." Since insufficient funds are available for research, "we must triage." He said researchers are trying to "enlarge the pie instead of re-dividing it" by seeking out new sources for grant money.

Dr. Modiano said dog owners could help research efforts by sending tissue samples and checking the box on the form allowing them to be shared. This will allow researchers to develop large sample sets for different studies.

Development of a National Canine Cancer Biospecimen Repository: The Canine Comparative Oncology and Genomics Consortium (CCOGC)

Matthew Breen, Ph.D.
North Carolina State University

Dr. Breen said, "In real estate, the maxim is 'location, location, location.' In research, it's 'samples, samples, samples.'"

"The sequencing of the canine genome has revolutionized what we can do," Dr. Breen said. Researchers' greatest need is a centrally based, well-described repository of tissue samples. Many cancer researchers collect their own samples; if those samples are not used, they are not used by anyone else either. In contrast, the consolidation of sample repositories will mean more rapid advances in canine research.

The CCOGC project began three years ago as a multi-disciplinary, multi-institutional community initiative by scientists across the nation, all sharing the goal of studying cancer in companion animals. CCOGC was incorporated in Maryland, and has also registered as a 501(c)(3) not-for-profit organization. Dr. Breen said it took 18 months to develop an operations plan, which included establishing a steering committee, selecting a board of directors, recruiting members, and forming a scientific advisory board.

CCOGC aims to collect 3,000 tissue samples over the next three years. Initially, it will focus on osteosarcoma, lymphoma, and melanoma, all diseases that occur in both dogs and humans. The organization hopes to collect 600 samples of each type of tumor, for a total of 1,800 samples. The specific type of the other 1,200 samples has yet to be determined, but the plan is to select four more types of cancer and collect 300 samples of each. Once the sample bank is established, Dr. Breen said, it will be self-sustaining. Users will pay for access to samples, and the money generated will be used to repopulate the bank.

The cost to establish CCOGC will be \$2.2 million over three years. CHF and the Morris Animal Foundation have each given \$250,000, which helped fund initial operations and establish the first three collection sites. The pharmaceutical firm Pfizer gave \$1.1 million, which moves forward the plan for six more sites.

The sample collection committee will ensure that both veterinary and human pathologists review every sample, and the scientific advisory board will respond to requests for access to samples, evaluating each request for the wise use of samples. Dr. Breen said researchers who want access to samples from CCOGC must have their study's funding already in place.

Currently, the project is “well on target with everything.” Three collection sites are already in use and plans are in place to establish between three and six more. The first three sites are at Ohio State University, Colorado State University, and the University of Wisconsin–Madison.

Sample collection begins when a site identifies an eligible patient, Dr. Breen said. CCOGC then sends a collection kit, and the sample is collected and sent to CCOGC, where it is cataloged and stored.

The storage facility, located at the National Cancer Institute in Frederick, Maryland, is safe, secure, and allows for real time inventory through a computerized barcode system. Dr. Breen said it follows the same standards as repositories for human samples, including the use of backup generators in case of power failure.

In the last six months, CCOGC has collected 68 samples. Dr. Breen said that if collections continue at the same rate, CCOGC might reach its goal of 3,000 samples in two and half years instead of three.

CCOGC is important because it is a shared, open access facility that allows multiple researchers to work on the same population, Dr. Breen said. Fewer individual researchers will need to collect their own samples, and the population of samples “will benefit many people and dogs for many years.”

Discussion

A participant asked how samples are submitted to CCOGC. Dr. Breen said those wishing to submit samples must be located in the same geographic region as one of the collection sites, because affected dogs must be seen by clinicians trained in specific procedures. While the application process for the next group of sites is under way, he said he could not reveal the contenders or any other details.

Understanding Cancer at the Breed Level

Rhonda Hovan
Research Facilitator
Golden Retriever Club of America

“Cancer has become a public disease in humans, but we haven’t broken the silence about cancer in dogs, and it is still considered a very private matter for some breeders,” Rhonda Hovan said.

Using Golden Retrievers as a model, she explained the importance of using survey data to understand how cancer affects a specific breed. For example, 60% of Golden Retrievers will die from cancer, but the average lifespan is still approximately 11 years, similar to other like-sized

breeds. Certain cancers also appear to be more prevalent, including hemangiosarcoma (HSA) and lymphoma.

“Cancer is not a single disease, but many diseases that share certain characteristics,” Hovan said.

Gathering accurate statistics on the disease is complicated, she said, because many owners report cancer by the location the tumor is found. Hemangiosarcoma (HSA) tumors, for example, can be found anywhere in the body because cancer cells can circulate throughout the bloodstream to other organs. No matter where the tumor is found, however, if the tumor cells are epithelial cells, then it can be accurately reported as HSA.

When cancer is suspected, Hovan recommended that breeders request pathology to get an accurate diagnosis, consider necropsy to determine the cause of death, and wait to report the disease until pathology or necropsy reports confirm the findings.

Cancer is a genetic disease, Hovan said, but it is not necessarily inherited. This is because genes are found in two kinds of cells: germ—both sperm and egg—cells, which affect the next generation, and somatic cells, which do not. Cancer is caused by mutations, or errors, in genes. Mutations can be found in either germ or somatic cells.

Most cancer in dogs is at least partially inherited. Even with mutations in a sperm or egg, however, Hovan said a puppy might not get cancer. A cell must go through many stages before it becomes cancerous. Puppies may be predisposed, but there are more factors that affect their chances of developing the disease.

Environmental risk factors, which can be random mutations, advance the dog a few more steps along the pathway to cancer. However, age appears to be the single, greatest risk factor for the disease.

“Any animal that is past its natural age of reproduction is at increased risk for cancer,” Hovan said.

Although animals have inherited mechanisms that favor good health, this theory of natural selection holds true only through the animal’s ancestral age of reproduction, which for dogs is about five to seven years. This is one of the reasons scientists consider cancer a natural part of the aging process, she said, and why cancer is rare in young animals.

Once a dog passes its natural age of reproduction, cancers that occur are often sporadic varieties that may be the result of unlucky mutations, and have no identifiable, inherited cause. Most cancers in dogs and humans are this kind.

Data collected on cancer in Golden Retrievers show the breed’s gene pool is widely divergent and its cancer rates are elevated worldwide. Immune-mediated diseases such as

hypothyroidism and allergies also are higher than average. Since cancer rates tend to increase when the immune system is compromised, immune-mediated diseases could signal increased cancer risk.

The breed's high rate of cancer, predominance of specific cancers, and high rate of immune-mediated diseases, taken together, indicates an inherited disposition for cancer in Golden Retrievers. Should this be true, Hovan said, the potential to reduce cancer risk through breeding should be examined.

This endeavor requires identified lines within the breed that have more or less cancer risk. No such lines exist within the Golden Retriever population, she said. If there are no dogs with a lower risk, there also are no available genes to mediate the gene pool.

Breeding in a longer lifespan might appear to be a way to extend the age of the dog before it gets cancer; however, this solution does not seem to work with any degree of consistency. The difference between dogs with middle-age-onset cancer and those with late-onset cancer—or those who may never get cancer—usually has more to do with random mutations and the environment than with heritable genes.

Although no good tools are available to help with breeding selection today, Hovan said studies are being funded by the CHF to identify genes associated with lower cancer risk.

In the meantime, modifying a dog's lifestyle, including keeping puppies and dogs lean and fit, can mitigate risk. Thin dogs have been shown to live more than two years longer than obese dogs, she said, and have a later age of onset for cancer. Supporting research indicates that feeding dietary supplements, including raw vegetables and fish oil, may reduce cancer risk. Limiting exposure to environmental factors linked to increased incidence of cancer, such as paints and solvents, asbestos, second-hand smoke, and certain herbicides is also helpful.

The link between cancer and other environmental factors such as x-rays is not as clear. Although fetal cells are most vulnerable to damage from x-rays, Hovan said, large lifetime studies of high-risk breeds exposed to standard doses of prenatal x-rays have not been done.

Age at neutering is another factor that can affect cancer risk. Breed-specific data collected on Golden Retrievers found that dogs neutered after one year of age lived longer than those neutered earlier. Again, Hovan said, the longer lifespan may translate into a later onset of the disease.

Scientists have recently learned that gene expression can be permanently modified prior to birth. A study with mice tested the theory by feeding pregnant females a diet supplemented with genistein, a soy protein. Their offspring had lower rates of diabetes, obesity, and cancer. However, breeding out disease in this fashion may have unintended consequences.

A similar situation occurred in 1976 when a research colony was formed in an attempt to eliminate hyperuricosura, a sometimes-fatal disease in Dalmatians that causes stones to form in the urinary tract. The disease has a recessive mode of inheritance, but all Dalmatians have two copies of the disease allele. Since no Dalmatians have a normal copy of the disease allele, Hovan said, it was impossible to breed out the disease.

To get around this, researchers introduced a Pointer—believed to be a distant ancestral breed of Dalmatians—into the Dalmatian population. After five generations, the resulting offspring were approximately 97% Dalmatian and 3% Pointer, had normal alleles and no hyperuricosura, and could be used to breed out the disease.

Unfortunately, she said, the dogs with the normal alleles always carried a spotting pattern that did not match the breed standard. It was discovered that the diseased allele was linked to correct Dalmatian spotting, and a choice was made to accept “stone forming” disease as part of what it means to be a Dalmatian.

Breeds, by definition, are closed gene pools, and therefore are under selection pressure to shrink. With each generation, Hovan said, desirable genes are selected for and less desirable genes are reduced or eliminated. Disease genes that are linked to desirable traits are selected for by default, and over generations become more common in the population.

Just as Dalmatian breeders had to decide whether to change the breed to reduce the risk of disease, others may be faced with similar challenges as more information is discovered about the link between DNA and disease. Hovan cautioned participants not to act hastily.

“As much as we are all going to eagerly await these new DNA tests that will tell us if our dogs are at risk or are not at risk for cancer, it may be healthier for our gene pools if we sort of sit back and let the whole picture emerge before these decisions are made.”

Hovan encouraged Parent Club members to donate samples from their affected dogs to help ongoing studies, and include that information on the Canine Health Information Center (CHIC) DNA Repository for future studies.

Discussion

A participant from the Afghan Hound Club of America asked when Golden Retrievers first come into season and the optimal age for sterilization. Hovan said Golden Retrievers first come into season near one year of age.

Another participant said more veterinary schools should encourage their students to do necropsies on dead dogs. She said it has been very difficult to convince veterinarians in her area to conduct necropsy on her dogs.

A participant questioned whether the fact that larger breeds age faster than smaller breeds was taken into account in the research. “Do cancer statistics go down for the breeds that age slower?” Hovan said she did not know, but that the topic bears further investigation.

Dispelling the Myths of Canine Cancer and its Treatment

Douglas Thamm, VMD, DACVIM
Colorado State University

One third to half of all dogs will develop cancer in their lifetime, Dr. Thamm said. This does not mean the disease is more prevalent than in the past, but rather that dogs are living longer because they are receiving better care, including vaccinations, nutrition, and supervision.

“[We are] allowing them to get to an age where diseases like cancer are being a problem,” he said. “There’s also more reporting of disease going on, so we’re much more aware of it.”

There appears to be a link between the incidence of certain cancers and exposure to environmental factors—including nasal tumors and passive smoke, lymphoma and certain herbicides, mesothelioma and asbestos, and bladder cancer and insecticides. However, Dr. Thamm said, these associations are either weak or have not been studied enough to make a solid conclusion.

Even if it cannot always be cured, cancer is treatable or at least manageable, Dr. Thamm said. Some statistics suggest that more than half of all tumors can be cured with surgery alone if the surgery is performed correctly. Furthermore, in those situations where a cure is not possible, “our real goal is to extend an excellent quality of life for whatever time is left.”

Dr. Thamm said he discourages owners and breeders from taking a “wait and see” attitude regarding their dogs’ cancer. Early diagnosis is critical. The larger the tumor, generally the harder it is to remove and more likely it is to spread.

Contrary to popular belief, neither biopsy using fine needle aspiration nor removal of the tumor will cause the cancer to spread. When surgery is performed, a complete histopathology should be submitted for proper diagnosis. Tumors that are removed completely are less likely to recur, Dr. Thamm said. Recurrent tumors are often more aggressive than first-time tumors and may be associated with a worse long-term outcome.

A study of dogs with mast cell tumor illustrates this point: 70% of first-time tumors did not recur if treated immediately with surgery and chemotherapy. When allowed to recur, the risk of death within one year increased to 90%.

Tumors sometimes recur because all the cancer cells were not removed during the initial treatment, Dr. Thamm said. The most aggressive tumor cells reside at the edges of a mass, so

taking tissue from around and under the tumor, or using radiation to kill cells that may be left behind, is critical to a successful outcome.

A pathology report will evaluate the tumor type and its histologic grade—how bad it is—as well as its margins. Margins that are clean indicate complete removal of the tumor; dirty margins are a sign of incomplete removal. A study of mast cell tumors showed that survival was 54 months when margins were clean, compared to 11 months when they were dirty. When the tumor was removed completely, Dr. Thamm said, 70% of subjects lived an additional 18 months. Incomplete removal meant only 15% survived that long.

Chemotherapy is used as a primary treatment for most forms of lymphoma, multiple myeloma, and leukemia. It also is used to help delay or prevent the spread of osteosarcoma, angiosarcoma, and certain mast cell tumors, among others.

Although dogs receive the same chemotherapy drugs as humans, the dosing is much lower in dogs, and fewer drugs are given at the same time, so the likelihood of adverse effects is considerably less. Dr. Thamm said fewer than one-third to one-fourth of patients experience significant unpleasant side effects such as nausea, diarrhea, and other intestinal symptoms. These can be readily treated, and in most cases, alleviated, by changing the dosage or switching to an alternate drug.

Other side effects may include lower white blood cell count and hair loss in certain breeds. Almost all chemotherapy is done on an outpatient basis. The risk of chemotherapy-related fatality is less than 1 in 200, Dr. Thamm said.

Owners and breeders need not be concerned about cross contamination, as few chemotherapy drugs are excreted longer than 48–72 hours after treatment. Dr. Thamm advised that gloves should be worn when handling feces, and pills should not be opened or crushed as this increases the risk of human exposure.

Age is not a factor during cancer treatment, Dr. Thamm said. The risks are no greater with older dogs, provided they are otherwise healthy. Treatment can vary from very conservative to very aggressive, depending on a number of factors, including finances.

It is impossible to guarantee how long an animal will live after chemotherapy. In studies of dogs with standard multicentric lymphoma, patients who received no treatment lived one month. Those that followed the full protocol of 16 injections over a period of six months averaged one more year of life, and 20% lived two years or longer. Overall, Dr. Thamm said, 90% of treated dogs showed improvement.

Radiation is a localized therapy used on sarcomas, mast cell tumors, and oral tumors, among others, to kill cancer cells left behind after incomplete removal of a tumor. It is used to shrink a tumor before surgery, as a primary therapy for certain tumors, or to ease pain or swelling. Radiation rarely has systemic side effects, Dr. Thamm said. Temporary side effects may include

a sunburn-like reaction at the treatment site. Treatment involves general anesthesia and a short hospital stay, with costs ranging from \$4,000 to \$7,000.

More information about canine cancer can be found on the Colorado State University Animal Cancer Center website at www.csuanimalcancercenter.org, or by calling the Center's free consultation line at (970) 297-4195.

Discussion

A participant said cancer remission in dogs is often misunderstood. Since one year of a dog's life is equivalent to 7–10 human years, one year of remission is a long time.

Another participant asked for Dr. Thamm's opinion on radioactive seed implant therapy. While still in its infancy in veterinary oncology, this treatment is being used on equine tumors of the head and neck, Dr. Thamm said. Similarly, radioactive iodine therapy—used on cats for some time to treat hyperthyroidism—is available, but only at a few centers across the country. This therapy renders the pet radioactive and requires a long hospital stay. Some studies on its use with dogs appear promising, he said, although finding a facility that offers this treatment can be difficult.

Optimal Nutrition for the Exercising Dog: Providing Nutrients That Make a Difference

Brian Zanghi, Ph.D.
Nestlé Purina Research Center

Health and well-being are optimized with proper nutrition. In his research on what constitutes proper nutrition, Dr. Brian Zanghi said he considered several groups of dogs, including sporting breeds that are very active in their daily life. He found that optimal nutrition results in dogs that are stronger, leaner, healthier, faster, and more alert.

Optimal nutrition is achieved through consuming foods that contain a proper balance of nutrients, he said. Nutrients are used as building blocks for the body, as signaling molecules, and for energy.

During exercise, a dog needs more energy and endurance, and therefore incurs greater caloric demand, increases protein metabolism, shifts nutrient utilization for energy in the muscle from glucose to fat, and requires more ability to metabolize oxygen. An unfortunate consequence of increased oxygen metabolism, Dr. Zanghi said, is the generation of increased oxygen radicals.

Most research conducted on the role of nutrients in optimizing exercise physiology has studied breeds on the far ends of the endurance spectrum—Alaskan sled dogs, which travel long distances, and Greyhounds, which run short sprints.

One study that focused on sled dogs compared the effects of high-fat and high-carbohydrate diets on exercising animals. Dietary fat is critical to promote fat metabolism and maintain high levels of fatty acids in the blood. Dogs that ate a high-carbohydrate diet had low levels of fatty acids in their blood both before and after exercise. Following exercise, those dogs had less ability to mobilize nutrients and promote aerobic metabolism, or higher endurance activity. When switched to a high-fat diet, subjects had higher levels of these substrates in their blood, and mobilization improved.

Nutrients can also shift the cellular makeup, as illustrated in a study of Alaskan sled dogs and Labrador Retrievers. The proportion of mitochondrial content in the muscle of Labradors on the high-carbohydrate diet was measured, Dr. Zanghi said. The Labradors were then switched to the high-fat diet. Shifting the diet increased the Labradors' metabolic capacity to slightly greater than the metabolic capacity of the sled dogs. This data suggests that endurance is not controlled solely by genetics.

The study also looked at maximum oxygen utilization with exercise: the higher the level of oxygen utilization, the greater the metabolic capacity and endurance. Once the Labradors were switched from the high-carbohydrate diet to the high-fat diet, Dr. Zanghi said, their metabolic and endurance capacity rose to that of the sled dogs.

High levels of dietary protein and fat promote increased mitochondrial synthesis for increased oxygen metabolism. Feeding a high-quality diet—high protein, high fat—will metabolically prime dogs to use these fuels during exercise. Switching from a performance food in the off-season may reduce the dog's capacity to utilize fat, he said, and reduce the animal's endurance.

Free radicals are a natural consequence of fat metabolism and using oxygen to generate energy. As endurance and aerobic metabolism increase, so do oxygen free radicals. Dr. Zanghi said proper nutrition could mitigate the negative effects of free radicals by providing antioxidant support, optimizing recovery after exercise.

Sources of oxidative stress during exercise are not only in the mitochondria but also occur in immune cells, where slight, low-grade inflammation can occur after bouts of strenuous exercise. Reducing inflammation or recovery time becomes very important, he said.

A recent study by Nestlé Purina evaluated antioxidants in the diet to see what kind of benefit they would provide to the exercising animal to reduce oxidative stress. Dogs in the placebo group received a regular performance diet with no antioxidant supplement. Dr. Zanghi said that within 24 hours of exercise, a considerable increase in the level of the oxidative stress biomarker—*isoprostane*—was noted. Conversely, dogs receiving the antioxidant *astaxanthin* showed a lower level of *isoprostane*, indicating optimal recovery.

Nutrients are also critical for skeletal health. Glucosamine promotes natural lubrication in joints. Omega-3 fatty acids improve joint mobility, Dr. Zanghi said, particularly in dogs with

osteoarthritis. Protein is needed to build and resynthesize muscles. Because an exercising dog has an increased level of protein metabolism, a higher level of protein in the diet helps keep the dog in a state of positive protein balance. Stress associated with physical activity could potentially be more detrimental in a dog fed a low-protein diet.

Optimal sensory perception is dependent on proper nutrition, Dr. Zanghi said. Studies showed that the omega-3 fatty acid docosahexaenoic acid (DHA), a nutrient found in mother's milk, helped in brain and vision development. Visual ability also has been enhanced in studies on dietary fat. Puppies fed a diet rich in DHA had greater sensitivity to light and darkness, and therefore could see better in low light.

Proper levels of dietary nutrients are very important to cognitive function. Blood glucose coming from glycogen stores in the liver is used primarily to support brain function, especially for staying focused and alert. With high-carbohydrate diets, Dr. Zanghi said, the body recognizes carbohydrates as its main source of energy. It stores them as glycogen in the muscles, but at higher levels than normal. When the muscle glycogen is depleted, liver glycogen is tapped, which leaves less blood glucose for the brain to use. The result is an earlier incidence of fatigue. With high-fat diets, the body depends less on storing glycogen to support muscle function, and therefore utilizes less.

Nutrients also have been shown to support optimal weight. In a longevity study of dogs, Dr. Zanghi said a diet containing the optimal ratio of protein to fat helped maintain lean muscle mass and healthy body condition. Even when fed higher quantities of food, the balanced nutrition in the diet helped promote a good body condition score.

Discussion

A participant reiterated the importance of proper weight management in dogs. Fat is not healthy, she said, and it is "up to us" to educate people.

Another participant expressed concern about the amount of nutrients her dog would receive if she fed it a less high-performance food. Dr. Zanghi replied that decreasing the quantity of food does not change the ratio of protein to fat in the diet. Animals need fewer calories in the off-season, he said; the key is to maintain lean muscle mass.

A participant cited an example of puppies fed a diet of 36% protein for 3–5 months. She described the puppies as thin, with broken-down and flattening feet. She asked whether this condition could be the result of the high protein diet. Protein has never contributed to improper growth, Dr. Zanghi said. Dogs should be able to tolerate up to 40%–50% protein in their diet with no deleterious effects. He said he considered it unlikely the puppies' condition would be due to elevated protein.

Canine Ophthalmology

Simon Petersen-Jones, DVM, Ph.D.
Michigan State University

Dr. Simon Petersen-Jones reported results from two projects that received partial funding from the CHF: a study of ocular melanosis in Cairn Terriers, and an effort to identify the genes responsible for progressive retinal atrophy (PRA).

With end-stage ocular melanosis, a dog is blind and unable to blink, has very enlarged eyes, and suffers from glaucoma brought on by severe proliferation of pigmented cells in the front chambers of the eyes. The condition progresses slowly, but the cells eventually plug up the outflow mechanisms that enable the eyes to drain into the bloodstream. The resulting canine glaucoma is different from the human disease and is difficult to treat effectively. Dr. Petersen-Jones said the age of onset in Cairns ranges widely: while some dogs are blind at age seven, others show early signs of the disease at 13–15 years of age.

Slides of early stage ocular melanosis show the root of the iris looking like a thickened doughnut, due to the proliferation of pigmented cells. As the condition progresses, specks of pigment show up in the front chamber of the eye, before gravitating down to occlude the drainage pathways. As secondary glaucoma occurs, the eyeballs enlarge, the eyelids bulge, and the dog goes blind.

Pedigree research to date has shown that ocular melanosis is an autosomal dominant condition, but the relevant gene has not yet been identified. So far, Dr. Petersen-Jones said, his lab has been working to exclude the genes that cause similar conditions in mice.

PRA is an umbrella term for a cluster of hereditary retinal dystrophies. Dr. Petersen-Jones also reported on achromatopsia—the canine equivalent of Leber’s congenital amaurosis, a disease that causes early blindness in young children. All three conditions affect the rod and cone receptors or the cells that deliver nutrition to retinal epithelial cells, causing significant loss of vision and often blindness.

PRA affects more than 100 breeds and is caused by a variety of gene mutations. It is similar to retinitis pigmentosa, a condition that affects one human in 3,500–4,000, and may be recessive, dominant, or X-linked. An eye affected by PRA is much more reflective than normal, and the blood vessels at the back of the eye are more attenuated. The condition begins with the loss of the rod photoreceptors. Dr. Petersen-Jones said a dog might react by refusing to go out at night or by showing poor night vision. As the disease progresses, the animal loses its cone-mediated daytime vision as well.

Dr. Petersen-Jones said his research has centered on Cardigan Welsh Corgis that are born without functional rods and gradually lose their cone vision. The relevant mutation affects the

gene that converts light entering the eye to electrical energy, resulting in a buildup of toxic substrates that rapidly kill the rod and cone receptors. The dogs' retinas are genetically normal, but they die without a supporting network of rods to keep them alive.

Leber's congenital amaurosis causes early blindness in three out of 100,000 children, for a total of 200,000 patients worldwide. The canine version of the condition is caused by a mutation of the *RPE65* gene that results in a protein deficiency, causing marked loss of day and night vision. Dr. Petersen-Jones said a major advantage for treatment is that the retina degenerates very slowly, in contrast to PRA, where the loss is much faster. Treatments for retinal degeneration include gene or drug therapy, transplantation using progenitor stem cells, and implantation of a "retinal chip" in an attempt to restore vision.

Theoretically, gene therapy can be used to introduce a new copy of a defective gene, or to slow down the degenerative process. Another option might be to introduce a therapeutic gene—a growth agent or neuroprotective agent—to keep the retina alive.

To introduce genes to the *RPE65* site, Dr. Petersen-Jones said researchers must inject the replacement gene into a virtual space behind the front of the retina. The treatment causes retinal detachment that clears after two or three days. However, a study several years ago showed that subretinal injection of a viral vector led to improved electroretinogram results and better retinal function in dogs. Moorfields Hospital in London, England, recently conducted the first human trials of the technique.

Dr. Petersen-Jones said further research is needed to determine how late genes can be injected to restore vision, and whether injecting the second eye will lead the body to mount an immune response that might jeopardize the vision improvements from an initial treatment. Time will tell how long the treatments last, he said, though the first dogs to receive gene therapy have retained their sight for several years.

Another treatment option for dogs is to introduce a synthetic version of the Vitamin A analog that is required for proper retinal function. The results of this therapy are not as dramatic, but some improvement in vision is still noted. Future therapeutic options could include a reservoir device to continuously inject a required drug into the retina, or an implant that gradually releases a retinal cell survival factor over a six-month period. Implants have been effective in rodents, cats, and dogs, and recently entered Phase I human trials.

Once the rod and cone receptors have died, Dr. Petersen-Jones said the only option is transplantation. However, progenitor retinal cells have shown some promise in mice, suggesting the possibility of a future treatment for advanced retinal degeneration.

American Kennel Club Update

John Lyons
Chief Operating Officer
AKC

Larry Sorensen
Director of Public Education
AKC

John Lyons congratulated the CHF for “another remarkable health conference” and gave participants an overview of several AKC programs.

The newest, AKC Veterinary Outreach, is a way of sharing information and ideas with veterinarians, veterinary technicians, and students. The program’s goal is to help the profession deliver the best possible care to dogs by providing background information for practitioners and their clients, supporting research, and funding scholarships for veterinary health professionals.

Scholarships are an important part of the picture. Lyons said five of the 12 students attending the conference were AKC scholarship recipients; he noted that scholarship funding “establishes a strong purebred information resource by producing future leaders in the pet health industry.” He listed several individuals whose achievements “demonstrate their dedication to their sport, and to the dog community as a whole,” and acknowledged Bayer’s donation of more than \$100,000 to fund 25 veterinary technician scholarships.

To be considered for a scholarship, a student must be enrolled in a veterinary or veterinary technician program accredited by the American Veterinary Medical Association. Winners are selected based on their academic achievement and breadth of experience, Lyons said. Veterinary students are selected by their schools; veterinary technician students apply through the AKC website.

The AKC also reaches out to the veterinary community through college seminars, and by attending veterinary conferences. With funding from Bayer, Lyons said, the organization offers a copy of *The Complete Dog Book* to every veterinary student in the United States.

AKC also runs an internship program to support leadership development and nurture careers for young professionals with a background and interest in the dog community. In seven years, he said, the program has hosted 39 interns who worked at 50 different positions in 29 AKC departments. Six of the interns were subsequently hired as full-time staff.

Lyons said a student must be enrolled in an accredited college to apply for an AKC internship, and must have completed at least one year of study before the internship begins. Winning candidates must have a competitive grade point average and a strong interest in purebred dogs.

The AKC Veterinary Network creates a bridge between the purebred dog clubs and the veterinary community, Lyons said. Participating veterinarians receive AKC materials for their practices, office listings on the AKC website, research updates, a newsletter, and new customer leads. So far, more than 3,200 veterinarians have joined the program.

Lyons announced that the AKC will move into its new, state-of-the-art operations center in Raleigh next year, and will donate 2,500 square feet of office space to the CHF. "We understand and appreciate the importance of the AKC Canine Health Foundation," he said, "and we are eager to contribute to its success in the future."

Larry Sorensen described the resources available to help purebred dog owners become community ambassadors for responsible dog ownership. Breeders and owners might think the time for advocacy is over when anti-canine legislation has been defeated, but "now's the time to be proactive," he said. "The idea is to prevent legislation through education."

Sorensen described the AKC resources available to clubs and individuals involved in public education or public education training. The public education department also organizes multi-club events to help recruit purebred dog owners into their Parent Clubs. AKC's goal is to hold 10 multi-club events across the United States next year, so "this is the time for your club to plan to hold an educational match in 2008."

Canine Legislation

Phil Guidry, JD
Legislative Analyst
AKC

"We are fighting for influence in the political process," Guidry said. "When one bad piece of legislation passes in any jurisdiction, everything we do can be called into question." Calling politics a "combative field," he said groups such as People for the Ethical Treatment of Animals (PETA) and the Humane Society are well funded, entrenched in the political process, and want to "end what we do as responsible dog breeders."

Canine issues are usually a low priority for legislative bodies, Guidry said. They most often arise as a response to a perceived problem. Breed-specific legislation usually follows press coverage of a specific incident, while neutering laws can arise from concerns about overpopulation. Other canine-related issues addressed by legislation can include dangerous dogs, animal cruelty, consumer protection, nuisance laws, and homeowner's insurance.

AKC's mantra regarding "dangerous dog" legislation is "It's the deed, not the breed," Guidry said. Members must reach out to educate legislators. "If you think for a second that your breed is thought of as friendly and has no chance of being targeted, think again." Movements to legislate often are based on "last year's bite statistics," with Labrador Retrievers and Golden

Retrievers usually the first breeds to be named. An extreme example of this type of law was passed in Italy, making the ownership of about 40 different breeds illegal.

Some “dangerous dog” laws are written in a way that does not adequately distinguish between threats to humans and to animals: dogs found to be menacing another animal can be classified as dangerous. A broad interpretation of this type of law could mean that any herding dog or retriever working in the field could be considered to be menacing another animal.

Animal cruelty has been in the news following the dog-fighting case involving professional football player Michael Vick. Guidry noted that those guilty of genuine cruelty, such as electrocuting or drowning a dog, should be “subject to the most harsh felony provisions possible.” However, some misdemeanor cruelty laws can have unintended consequences. According to one law, “if you leave your dog in your vehicle for five minutes while parking, you could be found guilty of cruelty from one complaint.” Cruelty laws should contain provisions regarding minimum care, and should provide due process and the opportunity to appeal.

The same is true of tethering issues. While jurisdictions have laws prohibiting tethering, Guidry said the AKC believes tethering is part of animal husbandry and can be done humanely. Cropping and docking is another issue that may come back in some jurisdictions.

Consumer protection laws should cover both buyers and breeders, he said. They should be explicit about how long dogs will be covered under the law and should include provisions for specific remedies, such as refunds or replacement. For the most part, current homeowner’s insurance laws are good because they follow the AKC’s mantra of “the deed, not the breed.”

Breeding restriction laws are “the biggie for us right now,” Guidry said. These laws are usually local in nature but sometimes are seen at the state level, and can take the form of requiring breeder permits that limit the number of reproductive events, usually to one per year. This can be hard on smaller breeders.

Guidry said the AKC played a role in defeating a California law calling for mandatory spaying or neutering of all dogs except for those whose owner’s had purchased a special permit. The proposed law, which would have affected 90–96% of California dog owners, was intended to address the problem of overpopulation, but was based on questionable statistics. Analyzing statistics is the key to determining if there really is a problem: California has such diversity that what constitutes a problem in one part of state is not a problem in another. The AKC has received information that the bill will be reintroduced next year, Guidry said.

Another such law is being considered in San Antonio, where a permit provision is to be voted upon to require owners of intact dogs to purchase a \$50 permit, plus licenses. Guidry called this “a *de facto* mandatory spay-neuter law,” noting that many poor and working class dog owners

might not be able to afford such permits. The law would also require owners to get a litter permit that would limit animals to one reproductive event per year.

“If there’s anything that scares me in this whole realm, it is the issue of animal guardianship,” Guidry said. These laws take animals outside property law and elevate them to “quasi-personhood,” and since they involve the taking of property, they raise serious Fifth Amendment issues. Similarly, if the government takes away the reproductive rights of breeding animals, they are substantially affecting the value of those animals. If governments want to mandate spay and neuter laws, he said, owners and breeders should be compensated for this loss of value.

Influencing the legislative process is a team effort that includes the AKC, state federations, local dog clubs, individuals and national specialty clubs. AKC’s Legislative Affairs department offers many tools to help educate and inform legislators and the public, including a newsletter, information on the AKC website, legislative alerts by e-mail, brochures, information packets and DVDs. AKC also can provide analysis of bills and ordinances, and supply experts to testify on canine-related issues. “If there’s ever anything we can do, we are readily available. Just call us,” Guidry said.

AKC can monitor state jurisdictions, but not laws at the local level. “That’s where you come in.” He urged dog owners, breeders, and clubs to be aware of and involved in the legislative process, and to get to know individual legislators.

“Political power is in your hands,” Guidry said. “Others may have money, but we have the votes.”

Is Canine Degenerative Cruciate Rupture a Consequence of Rheumatic Disease?

Peter Muir, BVSc, Ph.D.
University of Wisconsin–Madison

Dr. Peter Muir said that while US pet owners spend billions each year on the health care of companion animals, only a small fraction of that amount is spent on research. As a result, very little data exists on canine cruciate rupture, and researchers are just now beginning to understand some aspects of the disease. A 2005 survey done by the University of Iowa showed that spending on the care of dogs with cruciate ligament rupture represents a large portion of total canine health care spending, indicating that this is one of the most common and important orthopedic conditions in dogs.

Arthritis usually is classified into two forms: rheumatic or inflammatory, and osteoarthritis, with the latter usually considered more important in dogs. Canine cruciate rupture is similar to

ACL injury in humans, and is estimated to cause 20% of all lameness in dogs. Many large-breed dogs are affected with this condition, Dr. Muir said, and many dog owners do not recognize low-grade lameness. However, owners of hunting or agility dogs may see it earlier.

Dogs affected by cruciate rupture have prodromal lameness, and while the stifle or knee joint may appear stable, or exhibit only minimal instability, under radiographic examination it will show bilateral signs of arthritis. Ligaments may be frayed, Dr. Muir said, but are still attached to the femur and tibia. These conditions may persist for some time before a rupture finally occurs.

Histology of the collagen within the stifle joint of affected dogs suggests progressive mechanical overload of the ligaments, and radiographs show differences inside the stifle, including a roughening at the edges of the bone and a greater volume of fluid within the joint due to inflammation. Dr. Muir said this suggests the actual rupture and instability occur because of a pre-existing arthritis.

Affected stifle joints contain a mixture of inflammatory cells, including T lymphocytes, which are normally part of the body's defense against infection but also are associated strongly with immune-related rheumatic arthritis. "This cell is the central player in the development of persistent destructive inflammation," Dr. Muir said. Since the cruciate ligament gets most of its nutrition from joint fluid, the presence of inflammatory cells can cause irritation and have a profound effect on the tissue. This suggests that cruciate rupture is an oligoarthritis, which is defined as an inflammatory arthritis of four or fewer joints.

Traditionally, scientists have thought that inflammation and arthritis are the results of damage to the cruciate ligament, but Dr. Muir said his data suggests that the classical paradigm of relationship between inflammation and cruciate rupture is incorrect, and that synovitis actually precedes cruciate rupture and the development of instability. Over time, this inflammation may cause the ligament to become overloaded, leading to cruciate rupture.

While a normal canine stifle does not contain bacteria, analysis of the synovial tissue and joint fluid of dogs with cruciate rupture shows that the stifle joints of about half of the dogs contain bacteria similar to those associated with arthritis in humans. This mixture of bacteria is not necessarily pathogenic, like the bacteria that cause Lyme disease and subsequently lead to arthritis, but it could be an important trigger for the immune system.

While these immune reactions may lead to chronic disruptive inflammation in dogs that are genetically susceptible, Dr. Muir said that is insufficient data to confirm a cause-and-effect relationship. However, this is a very active field of research in both human arthritis and other diseases associated with chronic inflammation, such as Crohn's disease.

The majority of cruciate rupture dogs have degenerative rupture, but normal physiological events may allow their ligaments to remodel and adapt successfully. This may be what happens

in Greyhounds. However, most dogs are susceptible, and the combination of collagen degradation and trauma can lead to cruciate rupture during normal activity.

Goals for the management of cruciate rupture include gaining an improved understanding of the mechanisms that lead to cruciate rupture and the identification of biomarkers for prodromal oligoarthritis, which should lead to better tools to diagnose the condition while it is still in the arthritis phase. Current surgical treatments only address dynamic instability but do not help with passive instability or inflammation: while surgery may improve a dog's condition, it will not return the dog to normal. Comprehensive treatment is important and can be developed only with better understanding of the disease mechanism.

Discussion

A participant asked whether surgery is the only treatment option. Dr. Muir said it is too early to tell; while there is no question that joint inflammation is making dogs lame, treatments are also needed for other causes of lameness. The most effective current treatments offer pain relief, but do not reduce inflammation. There are many different drugs to treat inflammation in humans, but relatively few for dogs. Veterinarians want to move away from current surgeries that are ineffective, he said, creating pressure to conduct research that will improve medical and surgical treatment. While researchers are looking at tissue engineering, they should be looking more at ligament repair, Dr. Muir said.

Another participant asked whether Dr. Muir had reviewed other geographic areas where Lyme disease is not endemic, to compare research results. Dr. Muir said all his research data comes from Madison, Wisconsin and that it would be relevant to collaborate with a group from another region where Lyme disease is not endemic, or for other institutions to take up some of their research to see if inflammation and potential for treatment is similar or different. However, such work remains to be done.

A participant asked whether there is an obvious direction for health professionals to take, in terms of looking at human anti-inflammatory treatments and drugs to see if they will transfer to canines. "Yes, absolutely," Dr. Muir said. Cruciate rupture is a complex condition, and the current lack of information is a result of lack of funding.

Millions of dollars have been spent researching rheumatic arthritis, and yet the trigger is still not known. The condition is an economic burden to the country, Dr. Muir said, and determining how to bring more support to this program is very important.

Dr. Muir said research should continue to explore the idea of host/pathogen interactions. "In terms of therapy, what you say is right, and we're developing that conversation with industry and looking for new collaborative trials to find new drugs for dogs." Currently, the only available treatment for dogs is steroids. There is a pressing need for anti-inflammatory drugs to reduce inflammation without affecting the strength of collagen.

Nutrition and the Immune System: Advances, Implications, and a Case Study

Ebenezer Satyaraj, Ph.D.
Research Scientist
Nestlé Purina Research Center

Dr. Ebenezer Satyaraj said a healthy immune system is central to overall health and longevity. He encouraged participants to view the immune system not simply in anatomical terms, but from a functional point of view. These cells, tissue, and organs function “as a single entity in cooperation with other systems of the body, with the primary role of protecting the individual from two kinds of threats—internal and external.”

The gut is the largest immune organ, housing 60–70% of all immune cells in the body. Nutritional immunologists have an opportunity to incorporate agents into the diet that can “talk to” the immune system and influence the immune response. “It’s an exciting field that is evolving every day,” Dr. Satyaraj said.

Immune response is regulated by three elements: genes, life stage, and lifestyle. A puppy’s immune system takes several weeks or months to mature, so for its first 8–12 weeks, a puppy is protected by immune antibodies transferred from its mother. Maternal antibodies may not always be sufficient, Dr. Satyaraj said. If a puppy is raised in one region and then moved to another, for example, its antibodies may not be as effective against a potentially different set of diseases.

A dog’s life stage can also affect its immune response. There are times in the life of a dog when its immune system is not working at an optimum level. Among other challenges, the dog may not be able to fight infection. Lifestyle factors influencing immune response include stress, diet, exercise, and veterinary care, among others. Dr. Satyaraj said dogs are stressed when they are left alone, travel to a show, or move to a new home. Working dogs can be subject to fatigue because of their routine; fatigue has been shown to significantly lower the immune response in human athletes.

Unlike humans, pets must eat what their owners put in front of them. This presents a good opportunity to mediate the dog’s immune system by feeding a nutritious diet.

There are potentially four stages at which humans can interact with their pets’ immune system, Dr. Satyaraj said:

- The first stage is the basic feeding of a complete, balanced diet.
- In the second stage, nutrients such as vitamin D, copper, and selenium—all shown to nurture a healthy immune system—can be added.
- The third stage requires the owner or breeder to actively select completely natural

ingredients, such as probiotics and whey proteins, to strengthen and train the dog's immune system.

- In the final stage, the diet is tailored to the individual needs of the animal.

Dr. Satyaraj described how he evaluated, tested, and formulated a diet using whey protein, an immune-enhancing ingredient. First, he said, he conducted research and evaluated data from other studies, including safety implications. Whey protein is an excellent source of glutamine, the preferred fuel for both immune and muscle cells. Following a bout of strenuous exercise, glutamine levels drop. If the immune system faces an infection while glutamine levels are low, it may not be able to mount an effective response. Whey protein also is a source of glutathione, which helps build the body's defense against free radicals.

Next, Dr. Satyaraj said, he conducted nutritional trials, selected subjects, and developed the diet. The final step was to develop the product, including packaging and communications.

As part of the discovery phase, he said he conducted a pretrial test in which 48 Alaskan sled dogs, between two and six years of age, were fed one of four diets—one control and three test diets, named WPC-1, 2, and 3. The trial lasted 40 weeks. During a four-week pretest period, all four groups were given the same control diet. At the end of the pretest period, the test dogs were shifted to one of the three whey protein-enhanced diets. All subjects were given a canine distemper virus vaccine, Dr. Satyaraj said, and immune measurements were taken several times throughout the trial.

Systemic immune response was tested as a response to the vaccine. The immune response in all groups was boosted immediately following the vaccination, but it did not last long. The control group response dropped to its starting level. One test group showed a similar profile to the control group, but the other two test groups, WPC-1 and WPC-3, showed enhanced antibody levels that remained elevated throughout the trial. These animals were able to respond better and more strongly to the vaccine, Dr. Satyaraj said.

Local immune response was evaluated by measuring fecal IgA, an indirect measure of the activity level of gut-associated lymphoid tissue. By the end of the trial, the WPC-1 and WPC-3 groups showed higher levels of fecal IgA—and enhanced local immune status—than the other two groups.

The third test was designed to confirm whether the immune systems of these animals, having shown an enhanced response, were being over-stimulated. Levels of canine c-reactive, an acute phase protein produced by the liver in response to injury, infection, or inflammation, were measured. All groups had very similar readings, and all were within the normal range. The test showed the immune system in these animals was not hyperactive, Dr. Satyaraj said, but only responded when challenged. This is usually the hallmark of a healthy immune response.

The study also looked at fecal scores as a measure of balanced microflora. The animals were stressed by breaking their exercise routine. After two days of rest, the normal exercise schedule was resumed. Fecal samples were taken before and after exercise, and tested for stress-related shifts in gut microflora. The WPC-1 and WPC-3 groups showed nearly 80% similarity in their before- and after-stress patterns. Dr. Satyaraj said this result suggested the dogs had enhanced immune responses and were able to resist stress-related changes in the gut, a clear sign of a healthy dog.

Other biological measures, including body weight and blood profiles, were also tested. There were no significant differences among the groups.

Results of the study indicated that WPC-1 and WPC-3 provided enhanced systemic immune responses, enhanced local immune responses, no hyperactivation, increased gut microflora diversity, and greater stability of that microflora before and after the test.

These tests were used to launch a Nestlé Purina product called OptiStart Plus, currently on the market in Europe and South America. Dr. Satyaraj said he hoped it would be available to US consumers soon.

Discussion

Responding to a participant's question, Dr. Satyaraj said WPC-1 and WPC-3 are not commercially available, and are processed using a technology that even Nestlé Purina does not know. The participant also asked about the similarity of the dogs used in this trial. Dr. Satyaraj said he did not have this information, but that he would find out.

Another participant asked whether, if she wanted to feed an immune support enhancement diet and joint mobility diet simultaneously, she should buy two separate foods and mix them, or whether a multiple formula would be available. Dr. Satyaraj said he would take her suggestion back to the nutritionists who design these diets.

What Everyone Needs to Know About Canine Vaccines and Vaccination Programs

Ron Schultz, Ph.D.
University of Wisconsin-Madison

The three most important things to give a puppy, Dr. Ron Schultz said, are training, love, and vaccines.

The oldest and most common vaccine is the modified live vaccine (MLV). It is made by taking a disease-causing virus and altering, or attenuating, it to a non-disease-causing virus that is still capable of immunizing. Because natural infection or recovery from disease is the best kind of

immunity, these vaccines most resemble natural immunization, Dr. Schultz said. MLV is highly effective and generally very safe for the species for which it was developed.

Vector vaccine (VV) is similar to MLV, but produced by genetic engineering that usually incorporates DNA from more than one species of organism—recombinant DNA technology. In the case of canary pox virus, two genes from the canine distemper virus (CDV) are genetically engineered into a viral vector—canary pox—that protects against distemper.

“Genetically engineered vaccines are the wave of the future,” Dr. Schultz said. “[They] are the way most new vaccines will be developed, because we can better control what we do to the particular virus or bacteria, and we can make a very effective vaccine.”

To make MLV most effective, it must become more like the virulent form of the virus, and is then more likely to cause disease. Genetically engineered vaccines, on the other hand, have no such inverse relationship between efficacy and safety. Both MLV and VV are infectious vaccines, Dr. Schultz said. If the vaccines do not infect, they do not immunize.

The second most common form of vaccine is the so-called noninfectious variety. Inactivated or killed vaccines are this type. They are made by treating a disease-causing virus with a chemical or radiation to kill it. The organism should retain its important antigens, and, its ability to induce an immune response. Dr. Schultz said that sometimes the addition of an adjuvant, a substance that will enhance the immune response, is needed.

Another form of noninfectious vaccine is recombinant subunit vaccine. Recombinant vaccines are produced through conventional technology: grow the organism, inactivate it with a chemical, and then concentrate or separate out that portion of the bacteria that is most immunogenic.

The immune system is actually two systems in one, Dr. Schultz said. The innate immune system, also called natural or unspecific, is present from birth, protects against any substance, is nonspecific, and is not enhanced by prior exposure. Stomach acid, cough reflex, fevers, gut microflora, and even age are all examples of the innate immune system.

In contrast, the acquired—also known as adaptive or specific—immune system is highly specific or tailored to a specific organism, and enhanced by prior exposure. It involves a variety of organs, most especially the bone marrow where many immune cells are made, and the thymus. Dr. Schultz said the acquired immune system can be further subdivided into the humeral, or antibody mediated system, and cellular, or cell mediated system. In the humeral system, bone marrow cells contact antigens in the spleen, which produces antibodies. The cells of the thymus become differentiated into a variety of T cells with a variety of purposes.

Immunologic memory allows the immune system to remember the antigens or organisms to which it has previously been exposed. For example, following exposure to CDV, a dog’s immune system remembers for a lifetime that it has been exposed to the virus. A dog’s

immunologic memory of CDV, canine parvovirus (CPV), and canine adenovirus (CAV) guarantees long-term immunity against these diseases, similar to the long-term immunity afforded humans who have developed measles, mumps, or rubella.

“The number of cells and the kinds of networking that has to go on to produce that immune response is incredible,” Dr. Schultz said. “Fortunately, we don’t need to know much about this for the vaccine to work. The body itself knows what to do.”

In general, vaccines are designed to stimulate the acquired, or adaptive, immune system. At times they also can enhance nonspecific immunity. For example, intranasal kennel cough vaccine contains bordetella and canine influenza virus (CIV), with or without canine adenovirus-2 (CAV-2). The bordetella activates the nonspecific immune system within minutes of the dog receiving the vaccine, he said. Although it is not initially protecting the dog from bordetella, it is providing some nonspecific defense against various viruses.

Disease is dependent on the dose, the route of exposure, the virulence of the particular organism, and the host immune response. Dr. Schultz said innate immune response likely prevents infection with about 90% of organisms capable of causing infection. The other 10% are handled by the adaptive immune response, which typically is more efficient at battling viruses than the innate system.

All canine vaccines will stimulate both aspects of adaptive immunity—humeral and cellular. As is the case for the measles, mumps, and rubella vaccination, Dr. Schultz said, the most important factor for CDV, CPV, and CAV in dogs is the antibody. In a vaccinated individual, the antibody either prevents infection or limits it so the disease will not develop.

Duration of immunity comes through the immunologic memory cells, but the only way these cells respond is if the individual becomes infected. Dr. Schultz said it is now known that some of the cell-producing antibodies called plasma cells are long-lived. Instead of being present in the peripheral lymphoid organs like the spleen and lymph nodes, they are present in the bone marrow, and are programmed to continue to produce antibodies. If a particular cell dies, it makes a new cell also capable of making that antibody. This explains why dogs vaccinated against CDV, CPV, and CAV, and isolated to prevent any chance of getting infected with these diseases, still have the antibodies five to seven years later.

In general, Dr. Schultz said, duration of immunity is much longer for viruses than bacteria, and longer against a systemic disease than a local mucosal disease. Since CDV, CPV, and CAV are all systemic, viral diseases, the vaccine offers long-term immunity. On the other hand, bordetella’s contribution to kennel cough is more of a mucosal disease and a bacteria, so the immunity is short. MLV and recombinant VV provide a longer duration of immunity than any of the killed subunit vaccines.

Dr. Schultz described the role vaccines played in reducing the number of deaths associated with two diseases that have afflicted the canine species for hundreds, if not thousands of years, before a vaccine was made. The first vaccines were made in the late 1950s and early 1960s, he said.

The introduction of canine distemper vaccine led to significant reductions in the disease, though it has not been completely eliminated. The original vaccine for canine adenovirus was discontinued, after one in 500–700 dogs developed allergic reactions. However, the second-generation vaccine confers protection with no adverse reactions.

As a result, the United States has seen no cases of infectious canine hepatitis in domesticated animals in 15 years, except for three dogs in a San Diego shelter that had recently arrived from Mexico. The disease is still prevalent in countries and regions with lower vaccination rates.

Canine parvovirus first appeared in the late 1970s and “caused very, very high mortality before we really got a vaccine that worked,” Dr. Schultz said. Although the disease is far from being eliminated, immunization has led to a marked reduction in the number of parvovirus cases and resulting deaths.

Meanwhile, the past few years have seen a change in veterinary medicine’s philosophy of vaccination. Until recently, the predominant view was that every pet should receive every licensed vaccine multiple times at an early age, then at least annually through adulthood. Dr. Schultz said most vaccines were combined in a single syringe for the owner’s convenience, “not to worry about the animal itself being blown out of the water with 12-way, 14-way mombo combos.”

Practitioners assumed that even a vaccine that did not help would not hurt and, to this day, the labels on all USDA-approved vaccines specify annual boosters, regardless of the immunity they actually provide. “It is an arbitrary and capricious statement,” Dr. Schultz said, but the requirement is a part of the Federal Code and can only be changed by Congress.

Historically, vaccine recommendations came from a committee of the American Veterinary Medical Association (AVMA). However, after the American Association of Feline Practitioners published its first set of vaccine guidelines in 1998, AVMA left it up to specialty groups to do the same for other species. A group of clinicians and practitioners published the first canine recommendations in 2003, with administrative support from the American Animal Hospital Association (AAHA).

The guidelines were updated in 2006, and are available at www.aahanet.org. For the first time, the document lists the core vaccines—distemper, adeno, parvo, and rabies—that should be administered to every dog. “And we did mean ‘every,’” Dr. Schultz said. Although the United States probably vaccinates more dogs than any other country, its coverage is only about 50%.

With another 20–25% “we could, for all practical purposes, eliminate these diseases, because we would have so much better herd immunity.”

The main difference in 2006 was the recommended interval. The latest guidelines call for a last dose of core vaccine at 14–16 weeks, then at one year of age, then no more often than every three years. The exception is the rabies vaccine, which must be administered every three years. Optional vaccines for conditions like leptospirosis and bordetella are only recommended if an animal is at risk, but none of them provide more than a year of immunity. Dr. Schultz advised that if a dog requires one of the optional vaccines, “make sure it’s getting it often enough to get the protection you think it needs.”

For rabies, dogs require the first dose of vaccine at 12–16 weeks, then a year later, then every three years. A handful of states still require annual revaccination for rabies, but “that’s absolutely absurd. The law should be changed. There’s no immunologic or public health benefit,” and “you as a dog owner can help make that change.”

Since the minimum duration of immunity for the core vaccines is seven to 10 years, revaccinating every three years adds no benefit and increases the risk of adverse reactions. Those reactions are rare, but “it is not acceptable if an animal gets an injectable that is not needed and develops disease or dies.”

To determine the duration of immunity, the gold standard is to challenge with virulent agents. For an upcoming rabies vaccine study with which Dr. Schultz is involved, “the only way the results will be considered by the USDA and validated is by having a control group of dogs that will be challenged, and 80% of them must die from rabies for it to be a valid challenge. And then we will have to show the efficacy in the vaccinated group.”

He recalled a recent three-year study of a recombinant feline rabies vaccine that reported 100% protection in the vaccinated group, but only 79.3% mortality in the control. “That was unacceptable according to the Federal Code, so they’ve had to start the study all over again.”

Recent vaccine trials have shown long-term immunity of more than seven years for distemper and parvovirus, more than three years with a canary pox-vectored distemper vaccine, and more than seven years for canine adenovirus with the second-generation vaccine product. Where studies demonstrate three years of immunity, the next steps will be to test for five, then seven.

Dr. Schultz said it is critical for veterinary biological companies to endorse the three-year immunization schedule, as they have since 2004. “The practitioner really relies heavily on the company that supplies the vaccine. If anything goes wrong, they want to be backed up. If they need diagnostics, the company will generally pay for them.”

For the optional vaccines, it is critical to balance the odds of infection and disease against the risk of adverse reactions, while recognizing that treatment must be repeated annually and efficacy cannot approach the core vaccines. He emphasized that leptospirosis vaccines should

never be combined with treatments for viruses, and should never be administered before 12 weeks of age, because of the more immediate need for viral immunity and the impact of the leptospirosis treatment on a puppy's immune system.

Similarly, practitioners should avoid mixing viral immunizations with the vaccine for bordetella bronchiseptica. The treatments should be administered on opposite sides of the animal, so that they affect different lymph nodes.

Canine influenza virus jumped from horses to dogs in 2004, but Dr. Schultz said there was never any risk that influenza would become the next canine parvovirus epidemic. "Parvo is extremely environmentally stable, it's impossible to get rid of, it lives in the soil for at least a year or more, and remains infectious. Canine influenza is dead about the moment it comes out of the dog," so it seems to be most prevalent at Greyhound tracks, in shelters, and in some canine day cares or kennels.

AAHA specialists recommend against vaccinating for canine coronavirus or giardia. If coronavirus eventually emerges as a significant canine disease, most of the available countermeasures will not work, since killed vaccines do not induce mucosal immunity.

The issue of antibody titres has generated a great deal of confusion, Dr. Schultz said. Titres are often seen as a snapshot in time, but "what I want to tell you is that it doesn't matter with regard to distemper, parvovirus, and adenovirus. If there is any antibody that has been actively produced as a result of natural or artificial immunity, then the animal has immunologic memory and is immune." Younger animals should not be tested when there is any possibility of confusion due to passive immunity. Otherwise, the interpretation of test results showing low levels of immunity depends on which commercial test is used. For leptospirosis, antibody titres are indeed a snapshot in time.

It would be useful if puppies could be tested two or more weeks after their last dose of core vaccine to confirm immunity to distemper and parvovirus, Dr. Schultz said. Animals with no immunity could then be revaccinated and tested again after two weeks. That will not be possible without a more user-friendly, cost-effective, rapid test that could be used in veterinary offices.

Apart from revaccination, dogs often receive natural immunity through exposure to the core diseases. Dr. Schultz said he worries most about a lap dog that spends its life in its owner's apartment, before being placed in a kennel without being revaccinated. "That dog is likely to be in a pretty risky situation of coming down with severe kennel cough."

Although adverse reactions are rare, they are bound to happen. "When you inject something into yourself or your dog, there is always the chance of adverse reaction," he said. Reactions are considered rare if they occur in less than one in 10,000 animals, uncommon at a rate of one per 1,000 to 10,000, and common if they are more frequent than one in 1,000.

The biggest “rude awakening” on adverse reactions in veterinary medicine occurred in the late 1980s and early 1990s, with reports of injection-site sarcomas in cats. “No one ever suspected that a vaccine given to a normal, healthy kitten would end up causing lethal cancer, and if you don’t know, it can’t be, right?” Dr. Schultz said. “You’re convinced that a vaccine couldn’t cause cancer, but it did.” It turned out that felines lack the tumor suppression genes that would act on an injection site that became neoplastic through inflammation. The research in this area helped shift the philosophy that vaccines cannot hurt, even if they do not help.

Anaphylaxis is a much more common adverse reaction, but it has been known for 1000 years and veterinarians are trained to watch for it. Dr. Schultz listed a series of other adverse reactions—hives and facial edema, arthritis and polyarthritis, autoimmune hemolytic anemia, post-vaccination encephalitis or polyneuritis, seizures, abortion, congenital anomalies, embryonic or fetal death, failure to conceive, and transient immune suppression—in the five to seven days following a combined vaccination for distemper and adenovirus. He urged participants to report adverse reactions to veterinarians and vaccine manufacturers as a way of generating more reliable data on their occurrence.

Discussion

A participant asked whether a 15-month-old puppy should be immunized for leptospirosis along with its third dose of core vaccinations. Dr. Schultz said he would recommend administering the viral vaccines separately, following with the four-way leptospirosis vaccine at 17 and 20 weeks, then revaccinating six and 12 months later. If the dog is really at risk for leptospirosis, it should receive boosters every nine to 12 months.

The participant said she had never had her dogs vaccinated for leptospirosis in the past. “We’re going through a period of leptospirosis,” Dr. Schultz replied, “but lepto is no more a risk today than it was 20 years ago.” He said the disease is a concern in the participant’s home region of northern Illinois, at an incidence of one case per thousand or two thousand dogs.

A participant said mixed messages about titres have led to confusion for breeders and veterinarians. Dr. Schultz said it is more important to test a puppy two weeks after its last dose of core vaccine, rather than waiting until it reaches one year of age. However, for veterinarians in search of a practice management tool to ensure client compliance, his advice is “by all means do titres. Do not vaccinate. I have never seen an animal harmed from taking a blood sample. I have seen harm brought to animals by giving them vaccines that they do not need.”

With immunization as a management tool, “we have gotten the client to come in annually or more often, because everyone knew these vaccinations had to be given annually. We don’t want to take away that annual visit, because it’s critically important. So maybe the titre or the dental exam will do that.”

In reply to a question about parvovirus vaccines, Dr. Schultz traced the development of different parvovirus variants from the mid-1980s through 2005. Vaccines produced by the five major manufacturers are effective against all variants. However, there is a “window of susceptibility” when a puppy’s maternally derived antibody is insufficient to protect against infection, but still high enough to interfere with active immunity from the vaccine. Generally, that window has been reduced from 12 weeks to two, but it is still a factor.

“If you’re in an environment where parvo is a real threat, like a shelter, we’ll still see a few cases, even though we vaccinate every two weeks,” he said. “The virulent virus gets in before the vaccine can prevent the infection.”

He recommended against relying on some of the more obscure products available through catalogs.

A participant described her successful effort to eliminate giardia in her kennel, using a vaccine that veterinarians are hesitant to use. Dr. Schultz said the treatment is not needed for the majority of dogs, but that it makes sense in chronic cases where the benefit can be demonstrated. “If it works, by all means use it,” he said; the treatment might eventually find its way to his optional list if enough breeders can demonstrate its value. The participant said it is unfortunate that veterinarians are hesitant to use the vaccine where it is needed.

Another participant asked whether there is correlation between dogs being vaccinated at eight weeks and developing puppy stranglers. Dr. Schultz said he has seen reports to that effect, and that a vaccine can contribute to the disease it is designed to prevent.

A participant asked whether bitches should be vaccinated prior to breeding. Dr. Schultz said revaccination is not generally needed. If it is, it should take place prior to estrus.

Responding to a series of questions about vaccinations for toy breeds, he said body size is less important with biologics than it is with pharmaceuticals. For a vaccine, a Toy Poodle is as likely to need a full dose as a Saint Bernard. He said most of the smaller toy breeds have difficulty with the leptospirosis vaccine.

A participant said she had heard conflicting reports on the efficacy of rattlesnake vaccine. Dr. Schultz cited a veterinarian in Colorado who had found the vaccine effective, based on considerable experience with night rescues. He emphasized that the vaccine buys time, not immunity: according to one emergency veterinarian, dogs have died because their owners assumed their dogs were protected from rattlesnake bites and only sought treatment after it was too late.

A participant cited her veterinarian’s advice that parvovirus vaccine was unnecessary for a new litter of puppies born on a property that had recently been infected, as long as the puppies were dewormed. Dr. Schultz said an animal without intestinal parasites could still develop parvovirus, though dogs with both are more likely to get severe disease and die.

A participant asked whether the core vaccines could lead to a high incidence of autoimmune disease in specific breeds. Dr. Schultz said vaccines may trigger autoimmune disease, but they do not cause it. If dogs do not receive their core vaccines, he said, "They won't go on to develop thyroiditis. They'll die from distemper or parvo."

A participant who is a veterinarian challenged Dr. Schultz's statement that the rabies vaccine can be the most immunologically devastating for dogs. Dr. Schultz said the statement reflected practical experience. "Rabies is the most likely to cause an adverse reaction," he said. "That's what the quote is. And the reason is that it's a killed adjuvant vaccine."

Healthy Breeds and Breeding Recommendations

Jerold Bell, DVM
Tufts University

Much of the effort to create and maintain healthy breeds involves countering much of the conventional wisdom that breeders and veterinarians have developed in the past, Dr. Bell said.

Many breeders are committed to genetic diversity, and are concerned that closed studbooks will lead their breeds to doom and gloom. Some have responded by favoring assortative mating and outbreeding to the most distant genetic relations.

However, based on average 10-generation inbreeding coefficients for different breeds, Dr. Bell said breeding choices have no impact on genetic diversity. It is no surprise that breeds with very small gene pools have higher coefficients, and vice versa: The more important question is "what falls off the back, earlier on in a breed's formation." Inbreeding depression and hybrid vigor are not based strictly on homo or heterozygosity. "It really is all about knowing what deleterious recessives your breeds carry."

Genetic research is beginning to demonstrate the importance of missing some alleles, like those that predispose the Akita and some other breeds to autoimmune disease. The broader challenge for any breed is to work away from any deleterious alleles that are present. While some breeds can thrive with a higher inbreeding coefficient, others will do poorly due to a higher number of deleterious recessives.

Dr. Bell cited the popular sire syndrome as the biggest challenge to genetic diversity. When a great stud comes along, "his genes get spread far and wide across the gene pool." The breed loses much of its diversity, and the stud's deleterious recessives emerge several generations later. In addition to the "bottleneck effects" that result, the breed also loses the influence of quality dogs that should not have been pushed aside.

The clear conclusion is that "genetic diversity is breeder diversity," reflected in a healthy range of opinions on what constitutes the ideal dog. If some breeders decide to linebreed, some elect

to outbreed, and others orient their breeding programs to meet a variety of specific objectives, “that’s what will maintain the diversity in your gene pools,” Dr. Bell said. “It’s going to be the most important mechanism for maintaining a healthy breed.”

A crucial advantage for breeding programs is that “predictability is the hallmark of genetic disease,” Dr. Bell said. Problems can be diagnosed prior to onset, and breeders can intervene to prevent the expression of a genetic disease or trait and forestall its reintroduction to future generations.

But the familiar breeding recommendations are often less effective than they should be. A decision not to breed two dogs that have a common recessive gene will stop that gene from being expressed in the next generation, but will not prevent it propagating to future generations. Spaying and neutering a group of animals and starting fresh will be not only impractical or objectionable for many breeders, but impracticable, if it means learning a new set of genetic variables from the ground up. “If you wanted someone else’s lines you would have had them in the first place,” Dr. Bell said.

With that in mind, Dr. Bell said he orients his breeding recommendations to help breeders improve their lines “and not lose the ancestry you love, enjoy, and may have had in your home for 20 or more years.” It may mean deciding not to breed a particular dog, “but you can still carry on that line.” The end objective must always be to maintain and enhance the quality of the breed, which means looking at the entire animal and making decisions that will not limit genetic diversity.

To control genetic disease, the top priority is to reduce the frequency of dogs that carry defective genes. However, with tests available for more and more genetic disorders, the range of available animals becomes more limited. “All of a sudden, we’re not making our breeding decisions based on quality, on the one we really wanted to breed to in the first place, because we limited ourselves in the past by maintaining high carrier frequencies.” The breeders’ challenge is to select against carriers now, in order to open up more choices for future generations.

The situation becomes even more difficult with dominant genes in breeds with relatively small populations. The ideal is to replace an affected sire with one that is unaffected, particularly for late-onset diseases, but the option may not always be available. “It’s a difficult ethical decision to make.”

Dr. Bell emphasized that it will be impossible to deal with problems in specific breeds as long as they are kept secret.

Discussion

A participant asked how to phase out a condition that has 48% frequency in a breed with other common genetic diseases. Dr. Bell recommended collecting pedigree information and trying to

breed families with lower frequencies. "No one said it's easy," he said. The decisions are not easy or automatic.

Closing Remarks

Dr. Butherus asked participants to remember the sponsor "that made this whole thing possible," and the audience acknowledged Steve Remspecher of Nestlé Purina PetCare with a standing ovation.

"You are now ambassadors for this organization, and that's an awesome task," Remspecher said. "We ask that you carry forth our message and communicate all the information that's been shared with you."