

January 12, 2005

From: Senior Chair, Health and Genetics Committee
To: Board of Directors, Rhodesian Ridgeback Club of the United States
Re: 2006 Health and Genetics Committee Report

The 2006 Health and Genetics Committee Report is attached. The following motions are presented for the Board of Director's consideration. Please refer to the appropriate section of the report for a detailed discussion/description of each issue.

- 1. 2006 Health and Genetics Committee Membership.** By direction of the committee I move RRCUS approve the health and genetics committee membership for 2006 (Page 14 of this report).
- 2. Rhodesian Ridgeback Comprehensive Health Survey.** By direction of the committee I move RRCUS establish the Rhodesian Ridgeback Comprehensive Health Survey (Pages 15 of this report), at the approximate cost of \$1,150 in 2006 and approximately \$650 in subsequent years.
- 3. Rhodesian Ridgeback Health Website.** By direction of the committee I move RRCUS establish the Rhodesian Ridgeback health website (Pages 19 of this report) at the approximate cost of \$274.39 annually.
- 4. Degenerative Myelopathy and other Neurological Disease Sample Collection.** By direction of the committee I move RRCUS continue underwriting 50% the cost of necropsy (up to \$250) to confirm degenerative myelopathy **and** begin underwriting 50% of the cost of MRIs to help confirm epilepsy (up to \$600) at an annual cost of \$1500; **and** initiate a store-at-home DNA initiative that would allow us to collect DNA on multiple generations of dogs/litters at a cost of approximately \$280 per year (Page 21 of this report).
- 5. Continued Sample Collection for Research.** By direction of the committee I move RRCUS continue DNA collection for research, via cheek swabs at a cost of \$2.17 – \$4.34 per dog with the annual cost for swabs being approximately \$1521; **and** to approve funds for subsidizing confirmatory testing for megaesophagus at a cost of \$1500 (Page 22 of this report).
- 6. Budget request for 2006.** By direction of the committee I move RRCUS allocate \$7,200 as the working budget for 2006 of the Health and Genetics Committee (Page 24 of this report).

Cynthia Roethel

2006
RHODESIAN RIDGEBACK CLUB OF
THE UNITED STATES
HEALTH AND GENETICS COMMITTEE
REPORT

Prepared by:

Cynthia A. Roethel, Senior Chair
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Health and Genetics Committee

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*** Items require RRCUS Officer and Board of Directors approval.**

Aggressive Sample Collection for Research

As we head into 2006, the biggest research-focused priority for the Health & Genetics Committee is obtaining raw data -- valid, usable specimens.

Second only to having a solid research hypothesis, having valid specimens to test that hypothesis are the most important contributing factor in good research. Without them, there is no research – regardless of the amount of grant money available.

Getting valid, usable samples is not as easy as it sounds. Specimens can be unusable for many reasons, such as, mislabeling a tube or using the wrong one; specimens sitting at room temperature too long; keeping a specimen too cold; broken specimen containers, etc. Others reasons are a bit more complicated. If a researcher is studying, for example, canine epilepsy - there are certain diagnostic criteria that need to be met to ensure the dog is truly epileptic and not suffering from one of many conditions that cause seizures. In such a case, samples can be sent, but if there is no supporting documentation that shows the dog has epilepsy then the sample – as well as any contributed from family members -- is of little value. The same is true for deafness. Samples cannot be used without BAER testing. In the case of deafness BAER testing is needed on entire family groups. A researcher cannot take the word of the owner that one dog is deaf and another is hearing.

Specimens are critical – in many cases, more so than grant funding. This brings us to the next issue. While RRCUS is not a financially struggling club, we do not have the resources to sponsor breed specific research in every area of concern. **BUT** we do have the ability and the energy to collect samples that are “too good to refuse.” Laboratories do not shut down between grants. They are always up and running. When we find research in progress or even research that has been completed, we have been able to convince researchers to “piggyback” our specimens because:

- We do all the work for the researchers.
- The researchers get free, reliable specimens.
- The researcher has the potential to publish with little or no investment on their part, which impacts on their professional reputation, potential tenure and standing in the scientific community.
- The reputation of the university or institute is also enhanced which impacts admissions.

The point of this discussion is the RRCUS Health and Genetics Committee knows how to collect specimens and we have been able to bring out the best in Ridgeback people to help us get samples. By doing this, we have been able to create **breed-specific research** from grants designed to apply to all dogs. Here are three examples:

-- CHF Grant 2447 “Genetic Determinants of Susceptibility of Hypothyroid Disease in Dogs”. The first paper addresses hypothyroidism in Doberman Pinchers and addresses the data found in Rhodesian Ridgebacks. Because of the 24 valid and useful Ridgeback samples we submitted, the second paper will use that data to specifically address hypothyroidism in Rhodesian Ridgebacks. In fact we will be collecting additional specimens at the 2006 National Speciality for follow-up research as Dr. Kennedy wants a cross section of Rhodesian Ridgeback samples.

-- The University of California at Davis (UC Davis) has been thrilled with the quality and quantity of specimen collection for the ridge study. We have helped the researchers there expand the DNA-

submission form to include family history on other health conditions that UC Davis might explore in the future; this has already happened in the case of deafness. (See the Deafness Study – page 6) Dr. Neff and his associates are **NOT** dog people – they are not doing this for our benefit – they are under pressure as a veterinary research laboratory to research *disease* because they know that a marketable genetic marker for genetic disease is a potential financial resource and they want that resource. **BUT** they need us to give them *the correct* samples.

-- CHF Grant #324 “Molecular Genetic Characterization of Canine Non-Syndromic Deafness” specifically addresses deafness in Pointers. RRCUS did not sponsor this research. After Dr. Henthorn published that she found the marker, we were able to convince her to examine deafness in Rhodesian Ridgebacks when we were able to prove we could get meaningful specimens. Thanks to extremely conscientious breeders who had entire families BAER tested, we were able to provide blood specimens, clinical histories and consent forms on whole families of deaf Ridgebacks. The only costs associated with this, to date, have been administrative – postage, envelopes, labels, etc. With more work on the part of the Health and Genetics Committee and a minimal investment on the part of RRCUS, this effort will lead to a publication on deafness in the Rhodesian Ridgeback. (See the Deafness Study - page 6) What we get in return is **BREED SPECIFIC RESEARCH**.

Because it is in the best interest of the breed, we ask that the RRCUS Officers and Board of Directors aggressively support our efforts in sample collection for research.

Costs to date have been limited to \$2.17 - \$4.34 per dog – depending on the number of swabs sent. We do anticipate perhaps covering an occasional overnight shipping costs or perhaps subsidizing costs at National Specialities to facilitate specimen collection. (See the 2006 Budget Proposal – page 24.) There is a potential need to subsidize other high-cost diagnostic tests to validate specimens in the future for some sample collections. This is discussed under **Degenerative Myelopathy Initiative and Neurological Disease Sample Collection** (Page 21) and **Proposal for Continued Sample Collection for Research** (Page 22).

As you read the following report, keep in mind that most of the projects it delineates, current and future, are aimed at furthering one goal: reaching out to owners to obtain valid, usable samples on Ridgebacks with **confirmed** heritable disease and their extended families for ongoing or prospective research.

Hypothyroidism Research Results – A Direct Result of the 2004 RRCUS Sponsored Sample Collection

In 2004, RRCUS members and non-members alike submitted blood samples on their hypothyroid Rhodesian Ridgebacks. We sent 24 samples to the United Kingdom with the assistance of Dr. Jean Dodds, in support of a CHF-sponsored study on canine hypothyroidism. The chief investigator, Dr. Lorna Kennedy, has provided a lay summary of her results and it is presented with her permission in Appendix A. An outline of Dr. Kennedy's first publication from this research was presented as a poster display at the Canine Health Foundation Conference in September of 2005 and can be found in Appendix B. Note the late breaking Ridgeback data. There will be a second publication in 2006 that specifically addressed the Rhodesian Ridgeback data later this year.

Efforts are being coordinated to collect more samples at the 2006 National Speciality, as Dr. Kennedy needs additional samples on a cross section of Rhodesian Ridgebacks. Since these dogs will be having MSU panels done anyway, we will be collecting extra blood for this research. RRCUS costs will be limited essentially to shipping costs to MSU and to Dr. Dodds.

Deafness Study

Early in 2005 we learned of a genetic marker found in Pointers at the University of Pennsylvania for nonsyndromic deafness. The deafness sounded very similar to the deafness found in Rhodesian Ridgebacks. We contacted Dr. Henthorn who became interested when she learned that the Ridgeback has a Pointer ancestry. We submitted DNA blood samples, BAER testing results and clinical histories on 2 families.

RRCUS Costs: Less than \$30.00 in administrative supplies i.e. labels, envelopes, etc.

Dr. Henthorn has sent us a report of her findings. While the report itself is confidential Dr. Henthorn has authorized the release of the following information to the Board of Directors and the membership.

1. Rhodesian Ridgeback deafness and Pointer deafness are similar in that:
 - a. It is apparently non-syndromic and in particular, not associated with coat color.
 - b. Pups can hear at 8 weeks then lose hearing early in life
 - c. Deafness is bilateral
 - d. Deafness is inherited as an autosomal recessive trait (well established in Pointers and is consistent with available pedigree information in Rhodesian Ridgebacks).
2. Sample screening concluded that the Rhodesian Ridgeback **does not** appear to have the same gene defect as deaf Pointer dogs.
3. Dr. Henthorn and Dr. Steinberg, VMD, Dipl. ACVIM agree that even though the evidence seems to show that the deafness in Ridgebacks is not the same as the deafness in Pointers, it is important to publish a report documenting and describing deafness in Rhodesian Ridgebacks. *(Prior to H&G contacting Dr. Henthorn, deafness in Rhodesian Ridgebacks had not been observed by Dr. Steinberg, the veterinary neurologist who has been studying pointer deafness for many years. He has never seen a deaf Ridgeback and there is nothing in the scientific literature about deafness in Rhodesian Ridgebacks).* They suggest further studies which might include:

a. Examination of deaf and normal hearing Ridgebacks by University of Pennsylvania veterinary faculty, or through collaborations with a veterinary neurologist and a veterinary ophthalmologist to include BAER testing (requiring anesthesia), neurological examination and ERG (electroretinography, also requiring anesthesia). This would facilitate publication of Drs. Henthorn's and Steinberg's work and for the first time deafness in the Rhodesian Ridgeback will be a part of the scientific literature.

b. Perform a deaf x known carrier breeding or deaf x deaf breeding and follow the puppies with at least monthly BAER testing beginning at 4 weeks until some puppies lose their hearing. This study would be more valuable if histopathology of the inner ear of a deaf puppy could be performed. From a purely scientific standpoint this is a valid option but is the least desirable option from our prospective, as one of the pups would have to have histopathology which requires euthanasia at the University of Pennsylvania for immediate preparation of the tissues.

c. Continue to collect DNA and BAER results on deaf dogs and their relatives for a future whole genome scan or for more limited study that would involve examining genes known to cause deafness in humans (there are more than 50 genes known to be associated with non-syndromic deafness in humans).

d. Perform a partial or whole genome scan to identify markers that could be used for genetic testing. This would be costly and the chance of success would depend on the number of deaf dogs and families entered in the study.

The report from Dr. Henthorn will be sent to the 2006 Veterinary Review and Advisory Committee for their input on how RRCUS should deal with deafness in the breed. Their recommendation will be forwarded to the RRCUS Officers and Board of Directors via separate cover.

Since receiving this report the AKC/CHF has told us that Dr. Henthorn will be submitting an Acorn Grant to AKC/CHF to explore option (a) above.

Since receiving this report, we have located more families and several isolated deaf Ridgebacks. We contacted Dr. Neff, whose lab is very adept at genome scans. He has agreed to accept DNA swabs in anticipation of running these genome scans. We have started DNA swab collection on the families submitted to Dr. Henthorn as well as new families and isolated deaf Ridgebacks, and are having them forwarded to the UC Davis.

Allergic Dermatitis Study

This area of research is of *immediate* importance to Rhodesian Ridgebacks. Allergic dermatitis is our #4 health concern and often is debilitating and very frustrating for both the Ridgeback and its owner. Unfortunately this is an extremely difficult specimen collection endeavor. Difficult but not impossible.

Small amounts of blood are needed on the sire, dam and pups in a litter before the age of 6 months and then again at 12 and 18 months. These samples are needed to determine (1) if the predictor for allergic dermatitis works, and (2) at what age it manifests.

Since publicized in October 2005, we have received three inquires, of which we have had samples submitted on two litters, including the sires and dams.

Ridge Project

While this research project is still ongoing and many of its results are confidential, we are pleased to report that the laboratory at UC Davis has successfully mapped the region of the genome that governs ridge/ridgeless in the Rhodesian Ridgeback breed.

Currently, the researchers are using every means they have available to identify the exact gene responsible for determining whether a dog will have a ridge or not. Once the mutation is identified, UC Davis will work toward creating a DNA test that will tell breeders whether or not their dogs have the ability to produce ridgeless. The identification of a specific mutation will also pave the way for further ridge research, ideally allowing the researchers to identify modifying genes responsible for differences in ridge pattern, including crowns, fans and length.

Breed-community support of this project has been strong, and to date the number of Ridgeback samples in the UC Davis database is approaching 700 dogs. This represents a wide sampling of the breed, from perfectly ridged dogs to faulty ridged dogs to ridgeless.

Of the 700 samples, approximately 70 are ridgeless. UC Davis would like to add more ridgeless DNA to the database, and owners and breeders who have not yet submitted such DNA are encouraged to do so. DNA from ridged dogs is also needed and appreciated.

Recognizing that the breed faces a number of health issues unrelated to the ridge, and building on the excellent rapport we have built with researcher Dr. Mark Neff, DNA Liaison Denise Flaim revamped the DNA submission form in 2005 to include questions about two dozen health disorders found in the breed and presumed recessive.

In December 2005, UC Davis agreed to attempt to identify the specific gene that causes bilateral deafness in the Rhodesian Ridgeback. Sample submissions will begin in January.

Liver Nose Marker

Dr. Neff recently completed a small-scale pilot study on the inheritance of nose color in the Rhodesian Ridgeback. Based on the analysis of DNA from 10 liver nose Ridgebacks and one black nose, Dr. Neff has concluded that at least some, and possibly all, of the previously identified mutations in other breeds causes liver nose in Ridgebacks. This means that the same tests available for Labs and other breeds to determine if a dog is a carrier for the liver nose trait will theoretically work in Rhodesian Ridgebacks, i.e. the ChromaGene test offered by Vet Gen (www.vetgen.com/color.html). The Health and Genetics committee will explore the possibility of UC Davis' non-profit Veterinary Genetics Laboratory (VGL) creating a lower-cost test for Ridgebacks.

Cancer Samples

Since the Canine Health Foundation Conference in September 2005 and our subsequent push for samples, we have received 13 inquires on samples for cancer research.

- Osteosarcoma: 3 osteosarcoma samples submitted for research – one of which resulted in the establishment of an osteosarcoma tumor culture at the University of Colorado. At least 8 samples submitted to the Broad Institute/MIT as controls

for the osteosarcoma study.

- Lymphoma: 4 samples submitted for research to the Broad Institute/MIT.
- Mast Cell Tumor: 3 inquires made – one sample definitively sent for research to Dr. Cheryl London, at Ohio State University. The other two had not gone to surgery at the time of this report.
- Fibrosarcoma: 1 case forwarded to Dr. Bannik at the Animal Cancer Center at Colorado State University.
- Melanoma: 1 case of melanoma has been reported. The Ridgeback is undergoing testing. If further biopsies are required, an effort to coordinate a tumor culture will be made.
- Mammary Cancer: We have 2 dogs in a holding pattern who have been diagnosed with mammary cancer and had surgery. If the cancer recurs we will pursue tumor cultures.

Degenerative Myelopathy Initiative

In 2005 we have had three (3) intact spinal cord specimens from Ridgebacks presumptively diagnosed with DM submitted to the pathology department at Michigan State University for analysis. All three (3) were subsequently confirmed having DM based on the pathology results.

This has increased the number of Rhodesian Ridgebacks identified in the 2001 health-survey update by 33%, with nine cases initially reported.

DNA via cheek swab was collected on all these dogs. Swabs were sent to UC Davis as part of the ridge project but with annotations that these dogs were diagnosed with DM. Swabs are also kept in the owner's possession. See the further discussion on **Degenerative Myelopathy & Neurological Disease Sample Collection**. (Page 21)

We have received the receipts for two of these cases. RRCUS-subsidized share of the costs was \$303.00 for 2005. Our deadline for receipts expired December 31, 2005.

We currently have 3 cases pending for 2006.

Juvenile Cataract Study

In December 2005 the AKC/CHF announced the need for samples on dogs with hereditary cataracts. We contacted Dr. Cathryn Mellersh of the Animal Health Trust in the United Kingdom, who is working with the AKC/CHF.

She rapidly replied to our inquiry, telling us they have identified the mutation that causes hereditary cataract in three different breeds, and are interested to know if any other breeds carry the same mutation. To see if it is the same gene, she would need three or four samples from both affected dogs and carriers. If it turns out that Ridgebacks do not have the same mutation, they will need 20 samples of each to look for the specific gene mutation in our breed.

The Animal Health Trust is hoping to study hereditary cataract for many years to come as they just received fairly substantial funding to do so. Any samples sent to them now will certainly benefit their research.

They are asking for two cheek swabs per dog, a copy of the dog's pedigree to see if it related to any other dogs they have DNA on, and a copy of any eye certificates, CERF testing or letters from the dog's vet to confirm diagnosis, if available.

RRCUS costs include the cost of swabs and administrative costs only. We already have the three affected dogs and three carriers Dr. Mellersh needs to determine if the same gene mutation exists in Ridgebacks and they are "in the mail." If it is not the same gene, we are more than confident we will be able to collect the 20 affected dogs and 20 carriers needed to scan for the breed-specific gene mutation.

Status of Rhodesian Ridgeback Donor Advised Fund

Balance of the RRDAF as of 12/20/05: _____ \$21,790.34

Grant commitments for 2006 and 2007(See Grants Approved in 2005 for Funding in 2006 and 2007 below): _____ \$ 3,750.00

Available funds without further deposits: _____ **\$18,040.34**

Research Grants Supported in 2005

"Exploring the Genetic Loss of a Hallmark Trait; Ridgeless in the Rhodesian Ridgeback." Mark W. Neff, Ph.D. Center for Veterinary Genetics, School of Veterinary Medicine, University of California at Davis.

Acorn Grant approved by CHF for \$12,000. RRCUS required funding was 60% plus administrative costs: \$7776.

Funding of the Ridgeless Acorn Grant:

Donated funds:	\$1700
Funds raised at the 2005 National Speciality:	\$ 397
From the RRDAF:	\$5679

RRCUS deposits

1/28/05 - 2004 membership \$5 designation \$2,905.

1/4/2006 – 2005 membership \$5 designation \$2,505.

1/4/2006 – 2005 Speciality fundraiser for Ridgeless Acorn Grant \$397

Grants Approved in 2005 For Funding in 2006 and 2007

Grant #0373 Mapping Genes Associated With Osteosarcoma In Large Dog Breeds,
Principle Investigator: Dr. Kerstin Lindblad-Toh, Broad Institute/MIT; Dr. Evan Keller,
University of Michigan.

Total funding approved: \$211,184.

RRUCS committed \$2500/year for two years (2006 and 2007)

Grant #0257 Investigation of Predictors of Outcome for Canine Mast Cell Tumors

Principle Investigator: Elizabeth M. Whitley, DVM, PhD, Auburn University

Total funds approved: \$51,431.

RRCUS committed \$1250/year for two years (2006 and 2007)

Pending Grants

We have received the following grants from CHF for funding and these will be forwarded to the 2006 Veterinary Review and Advisor Committee for their recommendations on funding. The CHF Liaison recently learned from AKC/CHF there is a third grant from MIT on hemangiosarcoma that we have not yet received. This grant will also be forwarded to the Veterinary Review and Advisor Committee for their recommendations.

615: “Heritable and Sporadic Genetic Lesions in Canine Lymphoma” Jaime Modiano, VMD, PhD, University of Colorado Health Science Center, Matthew Breen, PhD, NCSU.

Lay Summary:

It has been apparent for some time that certain dog breeds are prone to develop certain types of cancer. Specifically, studies completed between the late 1960s and the early 1980s define relative risk of lymphoma for different dog breeds. Yet there was little progress since then to define factors that account for this risk. As part of ongoing programs supported by the AKC/CHF in our laboratories, we showed recently that the breed-specific risk of lymphoma extends beyond the simple disease condition to a predisposition for specific forms of lymphoma. More importantly, we showed there are recurrent chromosomal abnormalities that segregate with specific forms of lymphoma and that are more common in Golden Retrievers (with that form of the disease) than in other breeds, suggesting breed-specific profiles of genetic abnormalities will be found in canine lymphoma. To continue this work, we plan to use contemporary “array-based” technologies to identify genes that map to these regions and how they contribute to the disease. We anticipate that the results for this work will allow us to predict how heritable factors influence the occurrence of abnormalities in these genes, and will set the groundwork to identify specific genes associated with breed-dependent cancer risk.

Grant has been approved by CHF for \$302,294 over two years. CHF is asking RRCUS for \$5,000 over years.

632: “MicroRNAs and Canine Lymphoma” William Kessenberth, DVM, PhD; Ohio State University.

Lay Summary:

Lymphoma is one of the most common cancers in the dog. Current methods of classifying lymphoma neither explain nor predict its variable clinical behavior. Much of the progress in diagnosis, prognosis,

and treatment of lymphoma and other cancers in people have been the result of advances in “genomics.” Recently the canine genome has been sequenced, providing the opportunity to apply new genomic approaches to better understand and treat cancer in the dog. MicroRNAs (miRNA) are small non-protein coding molecules that have been implicated in humans as having an important role in cancer and a variety of other diseases. In this study, we will identify miRNAs using bioinformatics methods. We will then use miRNA microarrays to analyze normal canine tissues and canine lymphoma biopsies. These results (miRNA expression profiles) will be correlated with histologic, diagnostics and clinical parameters. The goals of this study are to identify canine miRNAs and their normal patterns of expression and to determine if specific histological subtypes of lymphoma are characterized by unique miRNA expression profiles, if specific miRNAs have prognostic significance, and to identify potential targets for future investigation and therapies. This study also generates new tools for future miRNA investigation in the dog.

Report of 2005 Budget and Expenditures

In 2005, we requested the following funding for 2005

DNA Inventory and Subsidize genetic testing: _____ \$1,600

Travel Expenses (CHF Conference): _____ \$2,000

Administrative Expenses: _____ \$ 500

Subsidizing Confirmation of Degenerative Myelopathy
Diagnosis: _____ \$1,500

Total budget requested for 2005: \$5,600

It was my understanding that only \$1,000 in travel was authorized to the CHF conference, leaving a budget of _____ \$4,600

The following is a report of actual expenditures for 2005

DNA swabs (2 cases): _____ \$ 450

Administrative expenses: _____ \$ 78.02

Betsy Pethick (Print): _____ \$ 130

Donation to the RRDAF in Dr. Pethick's name: _____ \$ 200

AKC/DNA Parentage Program subsidy
at 2005 Specialty for 33 dogs: _____ \$ 330

CERF report: _____ \$ 75

Travel expenses (two airfares for the CHF conference): _____ \$1,000 (approximate)

Subsidizing Confirmation of Degenerative Myelopathy
Diagnosis: _____ \$ 303

Total 2005 expenditures: \$2,566.02

Surplus of: _____ \$2,033.98

Proposal for 2006 H&G Committee Members

Request the BOD approve the following members of the 2006 H&G Committee.

Cynthia Roethel; 434-248-5018; houndscrest@earthlink.net H&G Committee Senior Chair; CHF Liaison; degenerative myelopathy project coordinator; epilepsy DNA collection coordinator; megaesophagus DNA collection coordinator.

Denise Flaim; 516-676-3398; revodona@aol.com; H&G Committee Chair; National Specialty Liaison; DNA Liaison; cataract DNA collection coordinator, deafness DNA collection coordinator.

Cynthia Willson, PhD; 919-309-7676; pingorarr@yahoo.com; Cancer Liaison - MCT; osteosarcoma; lymphoma; hemangiosarcoma, mammary cancer, brain cancer.

Melanie Behrens; 845-635-1489; melanieowl@aol.com; allergic dermatitis collection coordinator.

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“Rhodesian Ridgeback Comprehensive Health Survey” Proposal

(This proposal was submitted via E-mail to the 2005 BOD in December 2005. It is printed here for the benefit of the new members of the 2006 BOD).

What is the Health & Genetics committee proposing?

We are proposing that RRCUS adopt a software program developed by the American Spaniel Club as the Rhodesian Ridgeback Comprehensive Health Survey, which would allow dogs to be entered through the Internet or through regular mail submissions. The American Spaniel Club is donating the software **free of charge**. Costs associated with initial reformatting, placing it online, web site hosting and administration costs are estimated at \$1,150. Yearly costs thereafter are estimated at \$650 or less. RRCUS Health and Genetics Committee will edit the survey to make it Ridgeback specific.

Why do we need a new Health Survey and why should it be online?

1. Ability to update: Of utmost importance to the designers of the first Rhodesian Ridgeback Health Survey 10 years ago was that it be perpetual, so new dogs could be added and that it be confidential.

The survey worked wonderfully as a perpetual database. While there were assertions that the postmark on the envelope could indicate who submitted the survey, there is absolutely no identifying data on the survey whatsoever, making it as confidential as it could possibly be. However, the confidentiality, which was intended to encourage survey submission, has become a problem: Because specific dogs cannot be identified, updates are extremely difficult.

The survey was modified slightly to allow for updates for new submitters by having the submitter create their own survey number and make a copy of the survey before it was submitted. For those surveys already submitted, updates are only possible if the submitter recreated the data on the original survey -- i.e. number of dogs on the survey and identifying data for each dog, that included sex, date of birth, liver/black nose, spay/neuter status. When updates were received, Dr. Pethick, through heroic efforts, would go through surveys one by one until she found the matching survey.

We learned this endeavor was largely futile as few people made copies of the original survey they submitted, so accurately recreating the identifying data exactly as it appeared on the original survey was impossible for most submitters. Also, this approach was extremely time-consuming and was not the most productive use of our veterinarian-chair's time.

The online health survey we are proposing RRCUS adopt requires submitters create their own secure ID and PIN number so they will be able to access their dog's information. This will allow effortless updates, assure total confidentiality, and as a result encourage wider participation.

2. Ability to capture data on more Rhodesian Ridgebacks: An online health survey will allow us to capture data previously unavailable to us by making it easy and effortless for owners to submit information. Our current survey is unwieldy in terms of the long list of instructions, and an owner who is not a dedicated member of the breed community would likely not attempt to complete it. This is unfortunate, as we are striving to get as complete a picture of the breed's health as possible, and need submissions on all sorts of Ridgebacks.

By contrast, we have had great success in engaging non-RRCUS owners by using simple, straightforward links on the Internet. Alicia Franklin placed a link on the RRCUS home page asking visitors to email Denise Flaim if they are interested in participating in RRCUS health initiatives such as the DNA study. As a result, Denise receives about five inquiries a week from non-RRCUS members.

The American Spaniel Survey, which takes only 15 minutes to complete, is also an easily accessible, inviting survey that we feel would attract a significant pool of submitters from every corner of the Ridgeback world.

3. Ability to collect additional data: To date, we have largely collected data only on specific diseases found in Rhodesian Ridgebacks. There are other areas of critical importance where we should also be collecting data, including reproduction/whelping, adverse vaccination effects and toxin exposures. The online survey is extremely comprehensive and will give us insight into areas of the breed we have not been able to explore before, and will directly help our breeders and owners. For example: What is the average litter size for Rhodesian Ridgebacks and how does it change over the years? What is the prevalence of whelping difficulty? Is there a correlation to vaccination history and autoimmune disease manifestation? This comprehensive health survey includes everything we need and want in a survey.

4. Highly recommended: The RRCUS health survey coordinator, Dr. Katrina Viviano, works with Dr. Chet Thomas, DVM, PhD., at the University of Wisconsin who analyzes the American Spaniel Club data and it was he who recommended this survey for the Rhodesian Ridgeback. Dr. Viviano enthusiastically supports its adoption and as the health survey coordinator will analyze and publish the health survey data.

5. Record keeping for breeders and owners: The information breeders and owners enter on their dogs can be printed out for their own records. It can also be changed as the dog ages and new health findings emerge. In essence, it offers an in-depth snapshot of each dog's health history and status in every stage of life, which is valuable information that we often do not take the time to record as carefully as we should.

You can take a tour of the survey at: <http://www.lkhopkins.com/ascsurvey/index.html> .

To get an idea of the kind breadth we are proposing for the survey, please take a look at the statistical analysis done on data from the first 6 weeks after the Cocker Spaniel health survey was implemented: <http://www.asc-cockerspaniel.org/health/CBHSdec03report.pdf> .

What will happen to the data collected on the first health survey?

The data from our first health survey provided us with our first glimpse of the health of the breed. This data has been our resource in determining what research we need to pursue and what research we need to support. This data will always be available for us to evaluate our progress as it relates to improving the health of Rhodesian Ridgebacks. After thorough researching we have had to accept the fact that the original data cannot be incorporated with the new health survey. Without identifying data or another full proof way for *ALL* submitters to retrieve their original surveys the data would be meaningless in the context of the new survey – without the original surveys for submitters to reference, dogs could be entered twice or inadvertently omitted entirely. The key is having a method in place that allows submitters to enter information and then be able to access that information when necessary.

While it is unfortunate that we cannot incorporate the data from the first survey and continue to build on it, you have to remember the first health survey was designed 10 years ago and was the cream of the

crop approach to ensure data collection and confidentiality. But today we have a technological advantage that was not available 10 years ago. We are being given the opportunity to not only collect the data of the original survey but also environmental data that we may be able to correlate to disease.

What do we need to do to get this health survey up and running?

As mentioned above, the survey already exists and simply needs to be modified for our breed. The Cocker Spaniel - Comprehensive Breed Health Survey was years in the making and cost more than \$5,000 to develop. The American Spaniel Club is making this survey available to any parent breed club at no charge; the Leonberger breed club already has its version of the survey up and running.

How does the survey assure confidentiality?

When Ridgeback owners access the “Rhodesian Ridgeback Comprehensive Health Survey,” they will be automatically linked to a web site maintained by an independent agency, [Elements Software Engineering](#) (ESE). All health-survey data collected is stored there and is not accessible by any agency except ESE. Each owner will be asked to create his or her own ID and PIN number to proceed with data entry. Access to a dog’s information is only possible with the correct ID and PIN number. This is the exact same security used by bank debit cards, credit cards, etc.

No one – not the Health & Genetics Committee, not the RRCUS web master – can gain access to the ESE database. The company’s principal, Larry Hopkins, is not involved in the purebred fancy. He already maintains confidential health-survey sites for two other breed clubs, the American Spaniel Club and the Leonberger Club of America.

How does RRCUS obtain data from the Health Survey?

Before any data is sent to RRCUS for analysis, ESE strips any and all personal information that would identify an individual owner or an individual dog. Specifically, the owner's name, address and e-mail address, as well as the name and registration numbers of dogs, are removed. The generic information would then be sent to the health survey coordinator, Dr. Katrina Viviano, without any identifying details. ESE will forward the data as we request it. If we want data in order of disease prevalence – we can get it. If we want to know at what ages dogs have become hypothyroid, epileptic, etc – we can get it. If we want to know the prevalence of cancer in dogs on different types of diets – we can get it. The possibilities are endless. PLUS if we find a need to gather new information it can be added to the survey with approximately 30 minutes of programming time, i.e. \$20.

What does the Health and Genetics Committee have to do to get the online survey underway?

Once we receive BOD approval, we can begin to edit the survey to make it specific to the Rhodesian Ridgeback. We will use the same disease groups and diseases we outlined in our first survey plus incorporate some of the data the American Spaniel Club collects and will evaluate if there is other data we should take this opportunity to collect. We would like to have a prototype available at the 2006 National Speciality RRCUS for the membership to review and provide feedback. Our goal is to be ready to go online by the middle of 2006. It’s that simple.

What is this going to cost RRCUS?

Though the most expensive parts of the online survey – its development and testing – have already been paid for, there are nominal costs associated with this project as outlined below.

(1) The survey has to be altered to meet our needs and placed online.

(2) It has to be “hosted” by ESE on the web.

(3) There will be maintenance requirements. ESE will be available to deal with questions from the membership and forgotten IDs and PIN numbers, send data to our health survey coordinator for analysis, do backups, enter in manual data for those people who do not have internet access, etc, etc.

Larry Hopkins is willing to modify the survey for us, and has outlined the following costs:

Reformatting the survey and placing it online (one time cost): _____ \$500
(paid only when we are completely satisfied with the survey)

Recurring costs:

Monthly hosting fee is \$10/month. Yearly total is: _____ \$120

Biweekly backups are \$8 each. Yearly total is: _____ \$208

Office work: \$40/hour and actual expenses (postage, envelopes, mailers, etc).

Hourly rate would apply to modifications to the survey; stripping identifying data from the survey, “crunching the numbers” and forwarding data to the health survey coordinator; time spent manually entering data for those not internet capable; time spent communicating with any club members having problems, etc.

Estimate 6 hours of work: _____ \$240

Estimate actual expenses (postage, envelopes, mailers, etc): _____ \$ 82

First year total: _____ \$1,150

Subsequent yearly expenses:

Hosting fee: _____ \$120

Biweekly Backups: _____ \$208

Six hours of office work and actual expenses: _____ \$322

Subsequent yearly total: _____ \$650 or less

What happens if something happens to ESE or to Mr. Hopkins?

Mr. Hopkins has an escrow document lodged with a local attorney that contains his user ID and password to the web site. This document instructs the attorney to contact one of several local web-designers who can copy the Rhodesian Ridgeback database and all associated survey codes to a DVD. The attorney will then mail the DVD to another independent programmer pre-designated by RRCUS, or another RRCUS representative. The database is an ordinary Microsoft Access database with tables and field names documented so that anyone with a reasonable knowledge of Access can do the analysis and run reports, etc. If we wanted to continue the survey we could install the code on another server. We

would have to find someone familiar with Java script, Active Server Pages and Access to maintain the site for us.

The point is, the data would not be lost and the survey could continue.

What are the alternatives? The alternatives, none of which are optimal, include:

1. Continue with a non-electronic survey. This is very time-consuming for the committee; frustrating for users who have to shift through reams of paper; and does not give us an easy and effective mechanism for updates. Data collected between 1996 – 2001 is, for the most part, impossible to update.

2. Create our own survey. This would involve *hiring a programmer* to create an online health survey to our specifications; *Providing/purchasing* additional webspace for the health survey or host, and *locating a database manager* with no connection to Rhodesian Ridgebacks to monitor the database, organize the data we request, add in manual survey data and strip identifying data from the surveys, etc, etc. Essentially, this amounts to “reinventing the wheel,” as the American Spaniel Club has already covered this ground and offers their finished product **free of charge**.

It has been our intent to ask and answer any questions you might have concerning this project. If you have any **other** questions, or issues that we did not address please contact Cynthia Roethel at 434-248-5018 or at houndscrest@earthlink.net; and Denise Flaim at 516-676-3398 or at revodana@aol.com.

Rhodesian Ridgeback Health Web Site

What is the Health and Genetics Committee proposing?

We are proposing that RRCUS create a linked web site, www.ridgebackhealth.org, that would serve as a one-stop location for all things health-related in Ridgebacks for approximately \$275 per year.

Why do Rhodesian Ridgeback health issues need a separate website?

While this site would be RRCUS-owned and operated, and be accessed on the RRCUS home page via a prominent health link, we feel it is crucial that the site have its own address and identity for the following reasons:

- 1) It is clear that the more that the H&G Committee does to get/keep the Rhodesian Ridgeback a focal point in research endeavors, the harder we need to work at letting the membership know what we need. Repeated distribution of information in all media – magazines, web sites, email discussion groups and promotional material distributed at national specialties – is required to make every Ridgeback owner and breeder aware of how much is going on, and how they can contribute. But even that is not enough: There are so many ongoing projects and studies that the membership has difficulty keeping abreast of all of them. One of our goals for 2006 focuses on this pressing need: to make information readily and easily available for our members, and the Ridgeback community at large.

Frequent posts on RRCUS-members, RR-folk and other email lists, and articles in the club magazine, are helpful, but we still need a central place where owners and breeders can access useful, easy-to-find information about health issues facing the breed as well, researchers who are studying them, and the health survey.

- 2) Targeting and involving “lay” owners is an important goal for the health survey as well as all our health research. With its necessary focus on conformation, breeding and rankings, the RRCUS web site can be intimidating to some who are just casual owners. A web site that is focused solely on health removes some of those natural barriers and encourages the greatest amount of participation.
- 3) The CHF Conference gave us much to think about in terms of web sites. Just as you and I become frustrated with “buried” links, we learned that even requiring a user to perform an extra click minimizes the likelihood that the visitor to a web site will continue at that site. We want visitors to find health information immediately when they land on the site; having it a link away is simply one link too many. The rule of thumb in the internet industry is “no more than three clicks” to maintain a browser’s attention. Right now getting to the health survey is unwieldy for even members who have a fundamental insight to the RRCUS website. For the “lay” ridgeback owner, it is at a minimum intimidating if not impossible.
- 4) A separate web site permits us to associate key words with the site that are specifically and fundamentally health related. When an owner types the words “Ridgeback and cancer” in a search engine, we want the first result that pops up to be www.ridgebackhealth.org, an easy-to-find and remember address. The same goes for symmetrical lupoid onychodystrophy, or epilepsy, or any of a number of health problems that face the breed today. A web site only has a finite number of key words to attach to it, and health-specific problems and diseases should not take up all the key words on the RRCUS web site – that does not accurately reflect the parent club site’s content and somehow suggests that health disorders are a disproportionate focus in our breed community. But such specific and targeted key words are entirely appropriate for a specific Ridgeback health site so that we can reach as many owners as possible.

Who Will Design and Manage the Web Site?

Mike Teeling has volunteered as a member of the Health and Genetics committee in 2006 and will create and manage the Rhodesian Ridgeback health web site when approved by the BOD.

What is this going to cost?

We contacted Alicia Franklin to get a cost analysis on this project.

- 1-year domain registration_____ \$34.99
- 1-year web hosting_____ \$239.40
- Total** _____ **\$274.39**

Degenerative Myelopathy Initiative And Other Neurological Disease Sample Collection

What is the Health and Genetics Committee proposing?

We are proposing RRCUS continue underwriting 50% the cost of necropsy (up to \$250) to confirm degenerative myelopathy (DM) and begin underwriting 50% of the cost of a MRI to help confirm epilepsy (up to \$600) at an annual cost of \$1500; and initiate a store-at-home DNA initiative that would allow us to collect DNA on multiple generations of dogs/litters at a cost of approximately \$280 per year. This store-at-home program would allow us to capture the DNA associated with degenerative myelopathy, epilepsy and other late-onset neurological disorders. The cost of collecting a litter of 8 – swabbing 10 dogs would be approximately \$28. We estimate 10 litters per year (high estimate), with the total cost per year being approximately \$280. This cost is incorporated in the costs of swabs and administrative costs in the proposed budget for 2006. (Page 24).

The DM initiative we started in 2005 brought in three cases of presumed DM that were subsequently confirmed by Dr. Jon Patterson, pathologist at Michigan State University, which has increased our 2001 statistical incidence by 33%.

We had the opportunity to speak with Dr. Dennis O'Brien, who leads neurological research at the University of Missouri, at the Canine Health Foundation Conference. He explained that degenerative myelopathy and epilepsy are daunting diseases to research, due in large part to the complicated genetics and often advanced age of onset; in the case of degenerative myelopathy, by the time the dog is affected, any relatives, parents or siblings are usually unavailable. Having the DNA on just the affected dog is of little value. We **HAVE** to have DNA on near relatives and families. Dr. O'Brien was frank that it could take as long as 10 years to discover the genetics of diseases like DM and epilepsy and that is only **IF** they have the correct type and number of specimens.

As a result, our ongoing approach to degenerative myelopathy will be twofold:

1. We will continue to work to help owners and breeders with presumptive DM dogs to confirm the diagnosis by underwriting the cost of necropsy (up to \$250) to better understand the disease's prevalence and potential impact on the breed.
2. To maximize our ability to provide researchers with degenerative myelopathy **families** we will initiate a store-at-home DNA program for selected breeders who are willing to swab successive generations of litters and keep accurate, meticulous records on these dogs. By distributing DNA swabs to these breeders, we hope to develop a reservoir of multiple-generation Ridgeback DNA that, in years to come, could help us address the genetics of degenerative myelopathy along with other diseases that do not manifest until later in life – at a reasonable cost. This endeavor would be unrealistic if we used a DNA storage facility due to the cost, estimating 10 dogs being swabbed at each litter. This would be an additional cost of \$100 per litter if stored in a DNA facility.

To encourage DNA collection we want to **GIVE** DNA swabs (6 swabs per dog) to breeders who agree to:

1. Maintain detailed records on the dogs that had DNA collected. This means yearly follow-ups on litters and updates on the health survey and personal records. (We have had a rudimentary thought to somehow code these breeders in the new health survey database, but we have not fully explored that possibility or ramification).
2. Defer to the RRCUS Health and Genetics Committee chairs as to the use the DNA collected for bona fide research endeavors. We will establish a guideline as to how many of the swabs would be used for research and how many the breeder can keep for themselves for other genetic endeavors.

When there is the need for DNA for research or we have a “critical mass of samples,” the chairs will canvas the breeders participating in this program.

For those breeders who wish to collect generational DNA but who are not comfortable with the H&G committee directing the use of the DNA – the breeders will be charged \$.55 per swab. These persons will be approached to contribute DNA swabs for research but it will be up to the breeder to make that decision.

At a minimum one of the chairs will maintain records on the number of swabs held by these breeders. Much of this program is based on “the honor system.” While this seems tenuous, we urge the Board of Directors to understand that we have found breeders who are truly devoted to identifying degenerative myelopathy and epilepsy in the breed and to eliminating it. We know there are breeders who will cooperate with this study.

Cynthia Roethel has taken over the management of this program.

Continued Sample Collection for Research

What is the Health and Genetics Committee proposing?

We are proposing RRCUS allow our continued collection of DNA via cheek swabs at a cost of \$2.17 – \$4.34 per dog at \$1521 for the cost of swabs (costs is incorporated in the costs of swabs and administrative costs in the proposed budget for 2006 on page 24). **and** to approve funds for subsidizing confirmatory testing for megaesophagus at a cost of \$1500, Please see the *** items below concerning megaesophagus confirmatory testing.

In 2006, we will continue to aggressive pursue collection of specimens for research in the following areas:

- Cancer - Dr. Cynthia Willson, PhD, RRCUS Cancer Liaison
- Allergic Dermatitis – Melanie Behrens.
- Hypothyroidism – in conjunction with National Specialities – Cynthia Roethel
- Deafness – Cynthia Roethel and Denise Flaim
- Degenerative Myelopathy – Cynthia Roethel
- Cataracts – Denise Flaim

- Ridge samples – Denise Flaim

In 2006 we want to pursue collecting samples on the following:

- Megaesophagus***
- Epilepsy (See page 21)
- Other diseases were a unique opportunity to collect DNA presents itself

***Megaesophagus is not uncommon in Rhodesian Ridgebacks and is the #1 gastrointestinal disorder in the breed. We want to collect DNA on a litter of pups, where there is megaesophagus, and the extended family. We are confident we can find a litter but it is mandatory that disorders that can mimic megaesophagus be ruled out. Diagnostic confirmation is needed for valid DNA samples. We need to subsidize barium swallows on an entire litter of pups. This is necessary as we know that severity of megaesophagus can range from being subclinical to extremely severe. The clinical findings, including the results of barium swallow, provide the necessary data to make the DNA relevant. Reimbursement would be 50% of the costs barium swallow up to \$80.00 per dog. Our goal will be to subsidize 1 litter of pups this year and collect DNA on the pups and all available relatives.

Budget Request For 2006

In 2005 the projected budget request for 2006 was \$7,000 and was itemized as follows:

Cancer Conference Sponsorship*	\$2,500
Travel Expense (Cancer Conference)*	\$1,000
Administrative Expenses**	\$ 500
CERF report	\$ 75
Diagnosis confirmation in dogs with presumed Degenerative Myelopathy	\$1,500
Special Projects/Genetic testing subsidizing***	\$1,500

» Proposed 2006 Health and Genetics Budget «

Health survey	\$1,150
Ridgeback Health Web site	\$ 275
Administrative Expense**	\$ 500
CERF Report	\$ 75
DNA swabs 8 cases at \$225.07 each +/- 10%	\$1,801
Speciality Expenses:	
Dr. Mark Neff to address membership about RRCUS sponsored research: "Exploring the Genetic Loss of a Hallmark Trait; Ridgeless in the Rhodesian Ridgeback"	
No speaking fees. Air fare and hotel:	\$ 400
DM/Epilepsy initiative:	\$1,500
Subsidizing genetic testing/diagnostic testing***	\$1,500
Total:	\$7,201
Requested 2006 working budget for H&G:	\$7,200

* We removed the AKC/CHF Cancer Conference Sponsorship from the 2006 budget to divert funds toward sample collection. We already receive an update on the progress of cancer research at the AKC/CHF Canine Health Conference every other year, though admittedly abbreviated in comparison to the Cancer Conference. The information passed on at the Cancer Conference is groundbreaking and would enhance our Cancer Liaison's ability to interface with the cancer consortium; but with limited resources our money would be better used to orchestrate sample collections for cancer research.

**Administrative expenses include office supplies, specimen labels, shipping to and from the speciality location (microchip reader, files, forms, etc), specimen shipment and postage. Most persons arranging for specimen/tissue collection where specimens require overnight shipment assume the cost of the shipping. If shipping costs become a stumbling block we will reimburse up to \$50. We anticipate five overnight shipments per year at approximately \$50 each = \$250.

***Subsidizing genetic testing and diagnostic testing would include subsidizing genetic/health testing and collecting specimens for research at National Specialties to encourage the membership to participate as well as to subsidize barium swallows to confirm megaesophagus.

Projected Budget for 2007

Health Survey:_____	\$ 650
Ridgeback Health Web site:_____	\$ 275
Administrative expense:_____	\$ 500
CERF Report: _____	\$ 75
DNA Swabs: 8 cases at \$225.07 each +/- 10%:_____	\$1,800
CHF Conference travel expenses (2 people):_____	\$1,000
Special projects/subsidizing genetic testing at the speciality:_____	\$1,500
Subsidizing of High Cost Diagnostic Testing:_____	\$2,000
Total for 2007 _____	\$7,800

Appendix A

A Risk Marker For Canine Hypothyroid Disease

Dr Lorna J Kennedy

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Lay summary:

Canine hypothyroid disease is very similar to Hashimoto's Thyroid disease in humans, which has been shown to be associated with human Major Histocompatibility Complex (MHC) tissue types. A similar association with canine MHC genes in hypothyroid dogs would provide useful genetic markers for selective breeding to reduce disease incidence in pure-bred dogs.

In this study we have shown that the presence of a particular MHC allele or variant, called DLA-DQA1*00101, doubles the risk of a dog developing hypothyroid disease.

There is a clear genetic component to canine hypothyroid disease, particularly in closely inbred lines, and a number of breeds are thought to be more susceptible. Breed susceptibility may be related to the frequency of DLA-DQA1*00101 within each breed. Interestingly, several breeds that are not represented in our disease group do not normally carry this DLA allele within their populations.

The results suggest that while MHC is associated with increased risk in some breeds, it may have less influence in other breeds.

More dogs are being recruited into a follow-up study to investigate the effect of the MHC in different breeds.

Detailed report:

MHC genes are central to the regulation of the immune response including susceptibility to autoimmune diseases such as hypothyroid disease. Variation in immunity correlates strongly with differences observed in these genes.

The specific objective of this study was to investigate whether particular canine MHC class II alleles predispose to hypothyroid disease and the production of antibodies to canine thyroid antigens. We also wished to quantify the level of disease risk associated with different variants, and to clarify whether certain breeds are more at risk from the disease than others.

We collected DNA samples from 173 dogs with hypothyroid disease. Of these, a subset of 85 dogs have good clinical data, and can be confidently assigned with primary hypothyroid disease, based on the presence of anti-thyroglobulin antibodies together with other clinical signs. The other 88 dogs have incomplete clinical data (notably lacking anti-thyroglobulin antibody data), and therefore may represent a more heterogeneous group.

The affected dogs include only 42 different breeds, compared to our control set of 873 controls which contains dogs from over 70 different breeds. Some breeds are over represented in the patient group, such as Boxer, Doberman, Rhodesian Ridgeback and English Setter, while other breeds are not represented at all in the patients, such as Siberian Husky, Shih Tzu and Yorkshire Terrier.

We compared the total set of 173 diseased dogs with two different control sets of dogs: a) 267 breed matched dogs, and b) a large panel of 873 dogs (which has a representative breed distribution for the dog UK population).

We have compared the DLA allele frequencies of the patient groups with the control groups. In all these comparisons the same allele, DLA-DQA1*00101, was shown to be significantly associated with the presence of hypothyroid disease, see Table 1.

Table 1: Association of DLA-DQA1*00101 with hypothyroid disease

Patient group		Control group		X ²	OR	95% CI	P value
All affected dogs	105/173 (60.7%)	All controls	355/873 (40.7%)	22.7	2.25	1.59-3.19	<0.000002
All affected dogs	105/173 (60.7%)	Breed matched controls	122/267 (45.7%)	8.87	1.84	1.22-2.76	<0.003
Selected subset	57/85 (67.1%)	Breed matched controls	122/267 (45.7%)	10.94	1.97	1.32-2.94	<0.001

OR= Odds Ratio. CI = Confidence Interval.

The most stringent comparison of 85 dogs with putative primary hypothyroid disease with 267 breed matched controls gives an Odds Ratio of 1.97 and a p value < 0.001.

There is still a highly significant association of this allele with disease in the total patient group, suggesting that the group may not be as heterogeneous as we previously thought.

Several breeds are over-represented within this disease group, including Boxer, Doberman, Rhodesian Ridgeback and English Setter. In fact, it was difficult to find any Rhodesian Ridgebacks to use as controls, as it appears that this breed is highly susceptible to hypothyroid disease.

We have looked at each of these breeds separately with regards to the presence of DLA-DQA1*00101. It is clear that DQA1*00101 is raised in Dobermans, Rhodesian Ridgebacks and English setters with hypothyroid disease, but that it is not raised in Boxers.

These results suggest that MHC may influence the development of hypothyroid disease in some (e.g. Doberman, Rhodesian Ridgeback and English Setter), but not all (e.g. Boxer) breeds.

Interestingly, the breeds mentioned earlier as being not represented in the patient group, have low frequencies of DLA-DQA1*00101.

Appendix B



Association of Hypothyroid disease in Doberman Pinscher dogs with a rare Major Histocompatibility Complex DLA class II haplotype.

Lorna J Kennedy ¹, Heather J Huson ², Jayme Leonard ², John M Angles ³, Leslie E Fox ⁴, Janet W Wojciechowski ⁵, Annette Barnes ⁶, Christine Yuncker ², George M Happ ² & William ER Ollier ¹

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⁶ Veterinary Clinical Sciences, University of Liverpool, UK

Introduction

- Hashimoto's disease in humans is similar to canine hypothyroid disease
- Hashimoto's disease has been shown to be associated with the human MHC
- Some difficulty in definition of canine disease

Hypothesis: Is canine hypothyroid disease associated with the canine MHC?

Definition of primary canine hypothyroid disease

- Canine hypothyroid disease may be primary or secondary
- Only the primary disease is likely to be MHC associated
- Need to be able to distinguish primary disease cases
- To select this cohort of affected dogs we used strict clinical criteria, with an emphasis on the presence of anti-thyroglobulin antibodies

Materials: Numbers of dogs

- 27 Doberman Pinschers with primary hypothyroid disease
- 129 unaffected Doberman Pinschers
- Phenotypic and clinical data was also collected, including sex, age and full hypothyroid panel test results (TGAA%, TT4, TT3, FT4, FT3, autoT4, autoT3, CTSH).

Methods

- Sequence based typing
- Characterised alleles at three DLA class II loci
- Alleles assigned to haplotypes based on previous data

Numbers of affected and control Dobermans with each DLA haplotype

DRB1*	DQA1*	DQB1*	Number of affected dogs n = 27	%	Number of control dogs n = 129	%
00201	00901	00101	3	11.11	18	13.95
00601	00401	01303	25	92.59	110	85.27
01501	00901	00101	1	3.70	12	9.30
00601	005011	00701		0.00	2	1.55
01201	00101	00201	15	55.56	38	29.46
00601	005011	01303		0.00	6	4.65

Future work

- Sample a greater range of breeds
- Increase numbers of dogs in each group

Acknowledgements

This work was supported in part by grants from the American Kennel Club (No:2447), the Morris Animal Foundation (97CA09), the Doberman Pinscher Foundation of Michigan State University for the thyroid panel assays. We thank Lisa Boehmer of the United States for their help in collecting samples.

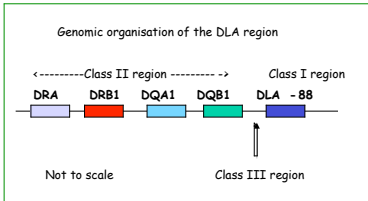
Take home messages

- Presence of DLA -DQA1*00101 doubles the risk of a dog developing primary hypothyroid disease
- May be a different mechanism in some breeds, e.g. Boxers, which do not show this association
- Some breeds, e.g. Siberian Husky, Shih Tzu and Yorkshire Terrier are not found in our disease cohort, and have low frequencies of DLA -DQA1*00101

the National Science Foundation (0346770). We thank Dr Raymond or samples from the UCLA Doberman colony. We thank Cynthia Roethel and the Rhodesian Ridgeback We also thank W. Jean Dodds of Hemopet, California for thyroid panel assays.

What is the MHC, and what does it do?

- MHC stands for the Major Histocompatibility Complex
- Every mammalian species has its own MHC
- The MHC includes many different genes
- The MHC controls the immune response, and is involved in response to infection susceptibility and resistance to disease rejection of transplants
- Two main types of MHC genes: class I and class II, with different functions
- Mammalian MHC genes are highly polymorphic (have many alleles)
- The canine MHC is called DLA (for Dog Leukocyte Antigen)
- Alleles are inherited from parents in groups or haplotypes



The Canine MHC, has three polymorphic class II genes: DLA -DRB1, DQA1 and DQB1

- Distinctive DLA -DRB1, DQA1 and DQB1 haplotypes have been identified
- High interbreed variation of allele and haplotype frequencies exists



Credit: UK Kennel Club Picture Library

Results

- Rare haplotype found at an increased frequency in the affected dogs compared to the unaffected dogs
- Odds Ratio = 2.43, Confidence limits = 1.19 - 7.61, p < 0.02.
- This haplotype has only been found in Doberman Pinschers and Labradors to date.

Late breaking news

- Latest study of 173 affected dogs shows that the association is with DQA1*00101
- 105/173 (60.7%) affected dogs had DQA1*00101 compared with 355/873 (40.7%) controls
- Odds Ratio = 2.25, Confidence limits = 1.59 - 3.19, p < 0.000002
- BUT may not be true for Boxers: see Table 2

Table 2	Doberman		English Setters		Rhodesian Ridgebacks		Boxers	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
DQA1*00101								
homozygous	1	0	10	13	17	5	0	2
heterozygous	14	4	6	13	8	6	4	8
not present	17	26	2	7	1	4	8	12
Frequency %	46.8	13.3	88.8	78.8	96.2	73.3	33.3	45.4
Total No dogs	32	30	18	33	26	15	12	22