3rd International Symposium

Increasing the life expectancy in Bernese Mountain Dogs

Burgdorf, August 10th, 2007

Ladies and Gentlemen
Dear Friends of the Bernese Mountain Dog

It is with great pleasure that I welcome you to Burgdorf, the location in which the Bernese Mountain Dog Club (BMDC) of Switzerland was founded one hundred years ago. I am happy to see that close to two hundred fanciers and breeders of Bernese Mountain Dogs from twenty-three different nations across the world celebrate this important anniversary with the BMDC of Switzerland by attending this symposium.

The main topic of this symposium is: "Increasing the life expectancy in Bernese Mountain Dogs". It supports the objective which the International Working Group (IWG) set for itself at the 2005 symposium, organized in Salzburg by the Schweizer Sennenhund Verein Österreich (VSSÖ), namely: To further world-wide cooperation in matters of health and longevity to achieve an average life expectancy of ten years (Objective 10) for our beloved breed. The 2006 symposium, organized in Como by Società Italiana del Bovaro del Bernese (SIBB), concentrated on scientific research carried out world-wide on the illnesses which most drastically shorten the life-span of our dogs, i.e. tumors and especially malignant histiocytosis. The present symposium will provide updates on the status of that world-wide scientific research initially presented in Italy by Catherine Andre, PhD, France, Matthew Breen, PhD, USA and Gerard R. Rutteman PhD, Holland. In addition it will provide an insight into how genetics influence life-expectancy by Gaudenz Dolf, PhD, Switzerland and into how the environment/behavioral health influences longevity by Urs Andrew Lüscher, PhD, USA. Mrs. Christel Fechler will inform us about practical steps and measures taken in the breeding program of the Schweizer Sennenhund Verein (SSV) Germany to increase longevity. My sincere thanks go to these speakers who are willing to share their specialist knowledge with us.

My thanks go to the VSSÖ, Austria and SIBB, Italy who have organized the previous two symposia. We were able to build our program as a logical continuation to the topics
that were discussed during the conferences they had organized.

The present symposium could not take place in its actual form, were it not for a very generous donation received from the heritage of Alberto Vittone. We remember Alberto in gratitude as one of the best friends the Bernese Mountain Dog ever had. Also, the Alberto Vittone Award, an annual financial award to support one or more projects to better the health and longevity of the Bernese Mountain Dog, will be distributed for the first time at this symposium.

Finally I would like to sincerely thank all the people who helped organize this symposium, especially Christine Irrgang Vogt and Silvia Brugger who carried the main responsibility for its organization.

I hope that this symposium will bring us another step closer to a better cooperation among all Bernese Mountain Dog breed clubs world-wide and to a better cooperation with the scientific community world-wide. Our beloved breed deserves nothing but the best. This may be a passionate statement, but nothing great has ever been achieved or created without passion.

Martha Cehrs

Martha Cehrs
President
Bernese Mountain Dog Club of Switzerland

3. International Symposium of the Bernese Mountain Dog Club of Switzerland

Date: August 10th, 2007
Locationt: Auditorium der Hochschule für Technik und Informatik
Pestalozzistrasse 20
3400 Burgdorf, Switzerland

Program

As of 8.15 Uhr	Arrival of participants	
9.00	Opening of the Symposium	Martha Cehrs
9.15	Life expectancy and genetics Is it possible to breed for longevity ?	PD Dr. Gaudenz Dolf University Berne, Switzerland
10.30	Discussion	
10.45	Break	
11.00	Life expectancy and environmental factors: Maintaining behavioral health	Dr. med vet Andrew U. Lüscher Perdue University, USA
12.15	Discussion	
12.30	Lunch break	
14.00	Practical breeding rules adopted by the SSV to improve life expectancy	Christel Fechler Swiss Mountain Dog Club (SSV) Germany
14.30	Discussion	
14.45	Analysis of the genetic causes of malignant histiocytosis of Bernese Mountain Dogs, progress report	Dr. Catherine André University Rennes, France
15.15	Discussion	
15.30	Break	
15.45	Molecular cytogenetics of MH in Bernese Mountain Dogs at NCSU, progress report	Dr. Matthew Breen, NCSU, USA presented by Dr. Catherine André
16.15	Discussion	
16.30	Investigation into the background of malignant histiocytic tumors in Bernese Mountain Dogs, progress report	Dr. Gerard R. Rutteman Utrecht University, Netherland
17.00	Discussion	
17.15	Progress report on activities of the International Working Group	Dr. Norbert Bachmann President SSV Deutschland, President B-IWG
17.30	Presentation of the Alberto Vittone Award	Antonio Indrizzi SIBB, Italy
17.45	Closure	

We reserve the right to make any necessary changes to the progam

Life Expectancy and Genetics
Is it possible to breed for longevity?

Gaudenz Dolf, PhD
Institute of Genetics, Vetsuisse Faculty, University of Berne, Berne, Switzerland

Life expectancy is the period of time an individual can be expected to live. The term can be used interchangeably with average lifespan and average longevity. Typically an individual takes some time to reach sexual maturity, then lives through a reproductive phase, and finally enters the complex process of aging. In humans aging is composed of several features:

- An exponential increase in mortality with age, that is the probability of dying at age 30 is much smaller than dying at age 70.
- Physiological changes that typically lead to a functional decline with age. Aging often is accompanied by unpleasant features such as loss of hearing or loss of eye sight.
- An increased susceptibility to certain disease with age. Such diseases causing death are heart diseases, cancer or neurological disorders, to name the most frequent ones.

The process of aging can not only be observed at the individual level but all the way down to the single cell. Factors influencing aging cannot easily be separated from those influencing longevity as in most organism aging is an integral part of longevity. In the following we do not pretend to be able to dissect this complex of longevity and aging but rather focus on the question whether there is enough genetics behind longevity in the Bernese Mountain Dog to breed for longevity. For this purpose we first should look at what is known in other organisms about longevity and aging.

There can be no doubt that environmental factors do have an impact on longevity as accidents or diseases can terminate a life. But there must be a strong genetic component as well. Otherwise it would be difficult to explain why a mouse should live no longer than 4 years but a bat of simillar body mass should live up to 34 years. The life span of the calico rockfish is about 12 years whereas its close relative, the rougheye rockfish lives over 200 years with no signs of aging. All salmons die shortly after reproduction but for the steelhead trout this holds not true. Although most of them die after reproduction some are able to return to the sea and reproduce another year. The female of the plaice, a flounder-like fish, continually grows and shows no sign of aging while the male ages and dies. These examples suggest that a genetic background must be rather complex. Despite these obvious differences in aging, the process of aging, at least in mammals, appears as a consistent process albeit proceeding at different rates.

Independently of the environmental conditions a mouse will age 25 to 30 times faster than a human being. Nutrition and exercise can make you live longer and attenuate certain age-related diseases, but you will not be able to live as along as a rougheye rockfish because humans age according to their genetic plan. This does not imply that aging evolved with a purpose that we easily could recognize. Also cancer has a strong genetic basis but it did not evolve with an apparent purpose. The greatest evidence in favor of seeing aging as a genetic plan is that many genes that modulate aging have been identified in model organisms such as yeast, a worm called *Caenorhabditis* elegans, fruit fly, and mice. In the GenAge database 61 genes are listed for yeast, 255 for *Caenorhabditis* elegans, 44 for the fruit fly and 47 for the mouse. All these genes seem to have an impact on aging and therefore on longevity. In the following table some of the genes investigated in these organisms are listed. The column “Life span” refers to the difference in the mean life span of mutants in comparison to wild-type individuals.

Species	Mutation	Gene description	Life span
C. elegans	age-1	human P(3)K homologue	65% increase
	daf-2	human insulin-receptor homologue	100% increase
	spe-10	unknown	40% increase
	spe-26	unknown	65% increase
	old-1	putative receptor tyrosine kinase	65% increase
	ctl-1	cytosolic catalase	25% decrease
	mev-1	cytochrome b subunit of succinate dehydrogenase	37% decrease
fruit fly	mth	putative G-protein-coupled receptor	35% increase
mouse	prop1	prophet of pit1	49% increase
	pit1	pituitary-specific transcription factor 1	49% increase
	ghr	growth hormone receptor	38% increase
	ghrhr	growth hormone releasing hormone receptor	23% increase
	plau	plasminogen activator, urokinase	20% increase
	shc1	src homology 2 domain-containing transforming protein C1	30% increase

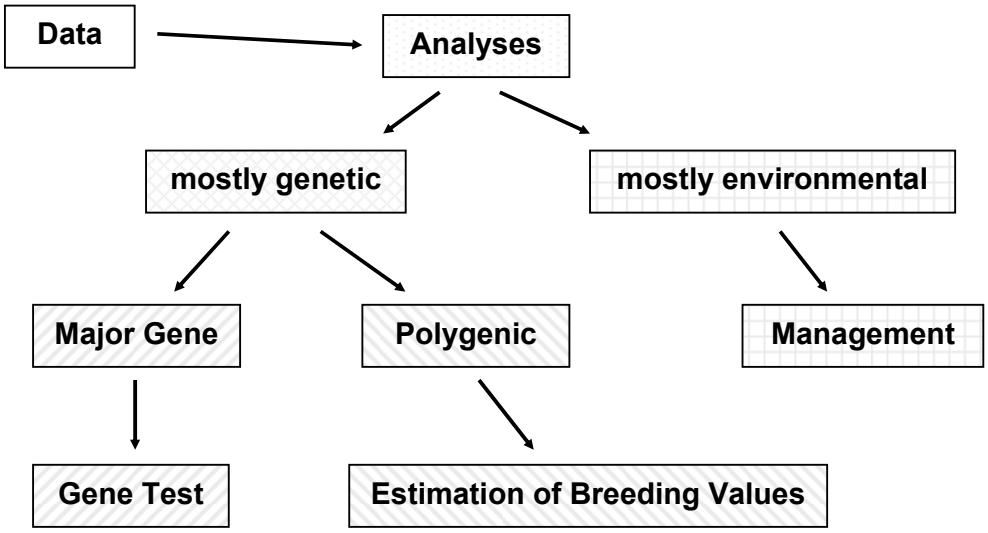
In humans the Cockayne syndrome type I seemingly leads to premature aging due to a recessive mutation in the ERCC8 (excision repair cross-complementing rodent repair deficiency, complementation group 8) gene. The Hutchinson-Gilford's progeroid syndrome is characterized by features resembling accelerated aging and caused by a dominant mutation in the LMNA (lamin A/C) gene. In Werner syndrome, caused by mutations in the WNR gene, the age-related changes of patients are remarkably similar to normal aging, only they occur at earlier ages. Further, apolipoprotein E has been implicated in human aging. The variant e2 seems to be associated with longevity, whereas the variant e4 is associated with early onset Altzheimer's disease. Besides the genes already mentioned, there are 15 other genes, among them interferon gamma, insulin receptor and tyrosin hydroxylase, which are significantly associated with aging in humans.

With the availability of the dog genome sequence it is now possible to directly identify all the genes, that have been implicated in the aging process in model organisms or humans, in the dog as well. For instance, ERCC8 is located on canine chromosome 2 covering the region 50'368'759 bp to 50'423'886 bp, LMNA on canine chromosome 7 covering the region 44'673'637 bp to 44'691'143 bp, and WNR possibly on canine chromosome 16 covering the region 36'120'426 bp to 36'257'633 bp. In the future this knowledge could be useful to elucidate the genetic background of longevity in the Bernese Mountain Dog. But would the knowledge of a single mutation in a gene modifying longevity allow us to better the present situation by breeding?

The fact that wild-derived mouse strains take longer to reach sexual maturity and live significantly longer than common laboratory strains suggests that longevity indeed can be altered by breeding. It is conceivable that in the Bernese Mountain Dog we unknowingly selected for gene constellations unfavorable for longevity. But if we can push longevity in one direction by breeding we should be able to reverse the process.

In order to increase the longevity we first have to know about the factors modifying life expectancy in the Bernese Mountain Dog. As in different species several hundred genes have been implicated in aging and, by extension in longevity, we have to assume that longevity in the Bernese Mountain dog is a rather complex trait too. Complex traits have in common that they include a genetic and an environmental component. The genetic part can be purely polygenic, but often one or more major genes can be identified. A major gene explains a large part of a phenotype but not the whole phenotype. In order to determine the nature of a complex trait a series of analyses is necessary. Variance component analyses will show whether environmental factors play a major role in the longevity problem. When including the relationships between the animals in the analyses, heritabilities,

maternal effects and genetic correlations can be estimated. Further, segregation analyses will reveal the nature of the genetic component if present. If a major gene can explain a substantial part of the variation in longevity we can go back to our list of genes involved in aging and try to narrow down that list in order to investigate one or a few such candidate genes. If a polygenic inheritance is most likely then the best way to tackle the problem would be to estimate breeding values for longevity.

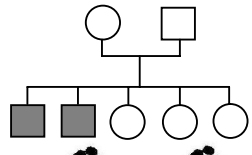


Up to here we have discussed possible factors influencing longevity and analyses able to reveal such factors. Once we know the factors we can develop strategies to improve longevity in the Bernese Mountain Dog. But before we enter this discussion we first have to think about the data needed to perform the analyses. Do we have the data necessary at hand or could the data be generated in a reasonable way? This is not a straightforward task as causes and effects of aging are not easily separated. For instance, the occurrence of hereditary diseases can interfere with longevity. Does, for example, malignant histiocytosis play a major role in decreasing the average longevity or would the dogs die anyway because of an altered genetic plan for aging? The problem boils down to the question of what information should enter the analyses to ensure meaningful results. The following list does not claim to be complete but it shows the basic information needed.

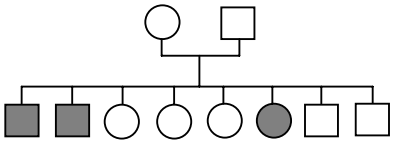
- Identity of individuals
- Pedigree
- Date of birth
- Sex
- Litter size
- Date of death
- Cause of death
- Kennel

For dogs registered with a kennel club information on identity, sex, date of birth and kennel is readily available. Also pedigree information poses no problem as the ancestry generally is well documented, often reaching back to the beginning of the breed. These data can be easily retrieved if stored in a computer database.

The litter size usually reported is the number of live registered puppies. But the true litter size often is larger as reported in the studbook, because there are puppies either stillborn or destroyed right after birth that do not appear in the studbook. This missing information usually can be obtained from the Kennel Club but often is incomplete. Consider a trait that is either present or absent at birth and the following pedigree:



Looking at this pedigree you immediately would suspect an X-chromosomal mode of inheritance of the trait as only the two male offspring are affected. Any decent computer program would come to the same conclusion. But now you learn that there were another three stillborn puppies in this litter. The stillborn female was affected but the two stillborn males were not affected.



Now you certainly would dismiss an X-chromosomal mode of inheritance in favor of an autosomal recessive mode of inheritance. Even if the phenotypes of the three stillborn puppies were not known they would allow for an autosomal recessive model. In a segregation analysis not only monogenic models but also more complex models including a polygenic component are tested. Therefore it is important to have the true litter size whenever possible in order to come up with the correct model.

The date of death is not available for most dogs, but a prerequisite if we want to find out about longevity. The same holds true for the causes of death. In most instances they simply are not available. But if we want to find out about the genetic background of longevity we need to have both, the date of death and the cause of death. These data are not easy to generate in the field as the majority of dogs of a breed belong to the non-breeding part of a given population. These dogs usually are kept as family or working dogs and their owners most often take not part in the activities of the kennel club. Therefore these owners cannot easily be enrolled in collecting data on a genetic disease even if it benefits their breed. With respect to longevity the real problem is that an owner should remember to report the date and cause of death at a time of mourning for a dear companion.

The cause of death rarely is established and only in context with accidents or a disease. The cause of death most often is based on the owner's opinion or a clinical diagnosis. Diagnoses based on necropsy findings virtually are not available as they cost additional money and often owners do not want to submit their dogs for a necropsy, especially in the case where the obvious reason for death was old age. But it is essential to know about the causes of death in order to find out whether we have a longevity problem or not. It is conceivable that by removing some of the causes of death the normal longevity could be restored. But it is also possible that some causes of death merely coincide with the natural time of death meaning that the genetic plan of dying indeed has been altered.

As we believe longevity to have a genetic basis, environmental factors should not play a crucial role. But as we do not know this for a fact it is worthwhile to evaluate possible environmental factors. For instance longevity could differ among different geographic regions which can be defined by the location of the different kennels. Or maybe dogs die more often in winter than in summer, which would be a seasonal effect defined by the dates of death.

Based on the prior knowledge on longevity it is safe to assume that we deal with a strong polygenic component which includes environmental factors and possibly one or more major genes. Getting a handle on such major genes even today with the availability of DNA microarray technology is a challenge and involves considerable time and money. Should the hypothesis of a strong polygenic component hold true the estimation of breeding values as basis for selection promises a faster and more cost-efficient way to deal with the longevity problem. It could be argued that by selecting for longevity we indirectly select against diseases which are leading to premature death. So all we would need would be the date of birth and the date of death to address the problem. But we have to keep in mind that the trait longevity could be correlated with other traits important in Bernese Mountain dogs. Therefore we cannot simply look at longevity but we have to monitor all the other traits as well in order to avoid improving longevity at other important traits' expense.

In conclusion, based on the knowledge on longevity in other species, it should be possible to increase longevity in dogs by breeding, provided the data necessary for the analyses is available or can be generated.

The statements on longevity in model organisms and humans are based on
http://www.senescence.info; Partridge L and Gems D, 2002, Nature Reviews Genetics 3, 165-175;
Finkel T and Holbrook NJ, 2000, Nature 408, 239-247.

PD Dr.Gaudenz Dolf

Institute of Genetics
Vetsuisse Faculty Berne
University of Berne
Bremgartenstrasse 109a
P.O. Box 8466,
3001 Berne, Switzerland

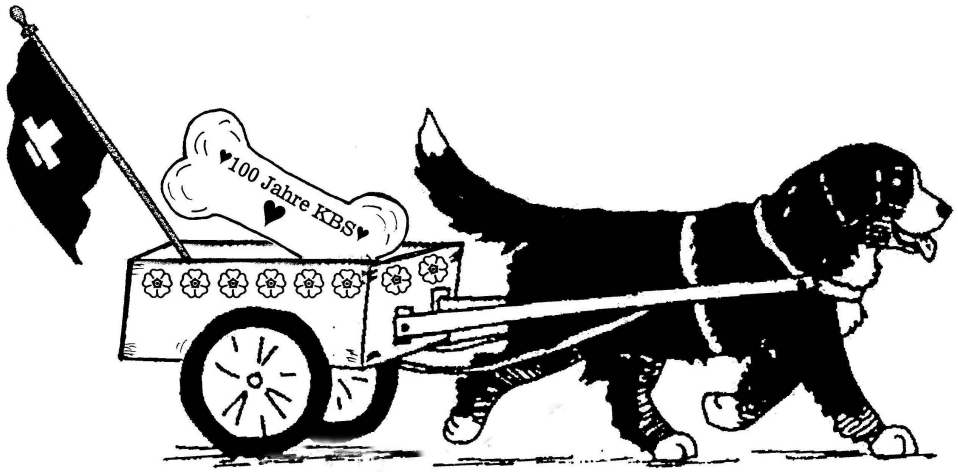
Phone +41 31 6312 320
Fax +41 31 6312 640
E-mail dolf.gaudenz@itz.unibe.ch

Studied Agronomy at the Swiss Federal Institute of Technology (ETH) in Zurich, majoring in Animal Production and specializing in Animal Genetics, graduated as Dipl.Ing.-Agr. ETH (1976-1980). Thereafter Assistant at the Institute of Animal Sciences at the ETH in Zurich (1980-1986) obtaining the Doctorate in 1984. Further studies at the University of Texas System Cancer Center, MD Anderson Hospital and Tumor Institute, Department of Molecular Genetics, Houston, Texas, USA (1986-1988). Since 1988 working at the Institute of Genetics at the University of Berne, and since 1999 Lecturer in Animal Genetics.

Research activities originally concentrated on Genome Analysis of farm and companion animals using molecular genetic methods. In the last 10 years there has been a strong emphasis on statistical genetics with the main focus on coupling und segregation analysis and genetic epidemiology. His research resulted in over 90 publications in peer reviewed scientific journals.

Member of the Scientific Advisory Committee of the Swiss Kennel Club, member of the Vetsuisse Research Commission of the University of Berne, member of the Advisory Board of Swissgenetics, Member of the Board of the Swiss Association of Animal Production, member of the Scientific Advisory Council of FUGATO (Functional Genome Analysis in Animal Organisms) of the Federal Ministry for Education and Research in Germany.

Also advisory functions for various breed clubs in Switzerland and Germany particularly focussing on breeding strategies with respect to specific problems.



Life Expectancy and Environmental Factors: Maintaining Behavioral Health

Andrew Urs Luescher Dr. med vet PhD DACVB ECVBM

Introduction

I have not found any publications that studied the relationship between stress and longevity in dogs. However, the documented detrimental effects of stress on physiological and behavioral wellbeing allow the conclusion that appropriate management of distress will contribute to welfare and longevity of our dogs.

Signs of Stress

Stress in dogs can be recognized by various physiological signs (Lindsay, 2000), such as:

- Pupillary dilatation
- Dilatation of blood vessels in the retina of the eye (change of eye color)
- Decreased pain sensitivity
- Decreased appetite and thirst
- Panting
- Increased perspiration on pads of the feet
- Increased heart rate
- Increased sympathetic arousal
- Frequent defecation, diarrhea
- Frequent urination
- Stronger startle and withdrawal reflexes

Behavioral signs include:

- Body language (ears back, tail tucked, body lowered)
- Conflict behavior (yawning, scratching, shifting eyes, scanning, pacing, etc.); compulsive disorder
- Escape behavior, scurrying, hiding
- Hyperactivity, hyper-reactivity
- Destructiveness, chewing licking
- Self-directed behaviors
- “Sexual” mounting
- Vocalization (high-pitched screaming, repetitive barking)
- Survival behaviors such as aggression (initially defensive, but may become offensive through conditioning with negative reinforcement)
- Inability to learn, focus
- Not accepting treats

Consequences of Stress

Stress, especially if frequent or chronic (non-avoidable) and anxiety have detrimental consequences:

- Atrophy of lymphatic glands and immunosuppression
- Changes in red-cell and white-cell blood values
- Gastric ulcers
- Degenerative effects on the brain that reduce ability to cope (less inhibition by higher brain centers)
- Increase in cortisol secretion
- More frequent and irregular urination, loss of house training
- Decreased appetite

Causes of Stress:

Stress can be caused as a result of genetic predisposition and the way we raise a puppy. Stress may also be caused by environmental conditions, including by how we manage the dogs, and by the nature of our interactions with dogs.

- Genetic predisposition: Some dogs are genetically predisposed to develop generalized fear or anxiety, sometimes very early in life. Others have a predisposition for developing specific fears, such as fear of loud noises (frequently late onset). Dogs that have been selected to be highly trainable are particularly prone to suffering from an inconsistent, unpredictable and uncontrollable environment.
- Early experience: A restricted early environment inhibits learning ability probably because of increased emotionality. Early removal from the litter has been shown in other species such as rats and cats to increase emotionality and anxiety (increased stress hormone turnover, decreased serotonin production). Severe disease in the first 16 weeks of a dog's life can also adversely affect emotionality (as evidenced by increased incidence of owner-directed aggression). Puppies that are not exposed to normal every-day stimuli between 3-14 weeks of age will be fearful and anxious as adults. If they are left in a restricted environment until 12-14 weeks of age, the chances of establishing normal responses are virtually non-existent. Psychological trauma in a fear period may result in generalized anxiety or specific fear.
- Traumatic experience: An adverse experience in a particular situation will cause anxiety and fear in that situation or to the aversive stimulus in the future (classical conditioning). Dogs can also suffer from post-traumatic stress syndrome.
- Environment: Unpredictable environment and social interactions, lack of consistent rules, loss of control over environment and over pleasant and aversive stimuli can result in learned helplessness.
- Motivational conflict: Strong opposing motivations resulting in conflict and conflict behavior. Frequently caused through inconsistent interactions and, especially, inappropriate use of punishment.
- Frustration: resulting form thwarted motivation, e.g., caused by social isolation, lack of exercise or lack of opportunity to investigate ("cabin fever").

Treatment of Stress and Anxiety:

Treatment needs to be specific to the cause, or to the specific anxiety-related behavior problem (e.g., compulsive disorder or separation anxiety). However, there are some general measures that can help alleviate stress:

- Consistent environment: Absolute rules and predictable and consistent interactions empower dogs by giving them the ability to control appetitive and aversive stimuli. Obedience training and command-response-reward type interactions contribute much to stress reduction.
- No punishment: Punishment is a very complex technique and difficult to use correctly. In addition, punishment is only appropriate for a very limited number of problem behaviors. Alternatives to punishment include, e.g., avoiding triggers, removing the reward, giving acceptable alternatives, and teaching an alternate behavior (response substitution).
- Exercise: Physical exercise has a modulatory effect on stress hormones, increases brain serotonin, boosts the immune system and reduces impulsive behaviors. Exercise off the property also allows for investigation of novel stimuli. Regular (twice-daily) walks off the property greatly reduce anxiety and alleviate behavior disorders that involve anxiety.
- Outlet for normal behavior: Investigation, chasing (predatory behavior), chewing, play, social interactions, etc, are normal dog behaviors that need an appropriate outlet.
- Environmental stimulation: All sensory systems, in particular the vomeronasal organ, give input into the limbic system that controls emotions. A very monotonous environment will increase anxiety (this may be why meal feeding is better than free-choice feeding in dogs), a more complex (albeit consistent and predictable) environment will decrease it. Commercially available pheromones (DAP) can be used to reduce anxiety
- Pharmacological treatment: Because of the limitations of and requirements for behavior modification techniques, temporary pharmacological intervention is often necessary to reduce the anxiety to a level where the animal can learn and behavior modification techniques can be effective.
- Specific treatment of behavior disorders: There are specific treatment protocols for anxiety-related behavior disorders such as separation anxiety, many forms of aggression, compulsive disorder, etc.

Prevention of Stress and Anxiety

Sound genetic makeup, optimal raising conditions, sufficient socialization and exposure, canine-appropriate management, feeding and exercise, a complex and consistent environment, predictable social interactions, competent training methods and restraint on discipline, the avoidance of traumatic experiences and good health status, all contribute to an emotionally balanced dog.

Genetic basis for emotionality: Researchers identified four personality traits in dogs, i.e., aggressiveness, playfulness, curiosity/fearlessness and chase-proneness, the latter three traits forming the super-trait shyness/boldness. Heritabilities of chase-proneness and aggressiveness have been shown to be moderate, but those for playfulness and curiosity/fearlessness are high. Therefore, selection of breeding animals for temperament should be successful given reliable and valid tests to measure it.

Complexity of Early Environment: An animal's central nervous system only retains its genetically predetermined functions if exposed to environmental stimulation, especially early in life. A restricted early environment will result in an animal with abnormal sensory perception that is emotionally unstable. In addition to emotionality, the complexity of the early environment also greatly influences learning ability. A restricted early environment may result in reduced trainability. It is therefore important to provide an interesting, stimulating early environment. In addition it is also important that the early environment be predictable and consistent. If not, the animal will learn that its behavior has no impact on what is happening around it, and it will be in a state of learned helplessness. Such animals are exceedingly hard to train later on.

Effect of Neonatal Stress: Some degree of stress (e.g. handling, cold temperature) in the neonatal period of dogs may accelerate growth, reduce emotionality, and increase resistance to some diseases. Handling sessions from the first days of a puppy's life are therefore recommended (about 3 minutes/day are sufficient). In addition, puppies may be removed from the nest (best while someone else walks the mother) and placed on a cool vinyl floor for a brief time (30 seconds) before being put back into the warm nest. If done in the first few days after birth, this is expected to result in reduced behavioral and physiological reaction to chronic stress, an increased physiological reaction to acute stress, and reduced emotionality of the adult dog. Chronic stress is caused by unavoidable and long-lasting aversive conditions. Since they are unavoidable, the stress reaction does not result in coping, and just drains the animal's resources. Such chronic stress causes stomach ulcers and other impairment of health. A strong reaction to acute stress, however, is desirable. If a grand piano falls from the sky directly towards you, it may save your life to mobilize all your resources to get away from there. So both a reduced reaction to chronic stress and an increased reaction to acute stress are beneficial.

Socialization and exposure: The socialization period of dogs begins at about 3 weeks and extends to about 12 or 14 weeks of age. Socialization to dogs and to people has to occur during this time (it needs to be continued thereafter). If this opportunity is missed, the puppy will most likely always be fearful of dogs and/or humans. During the socialization period, the puppy should also be exposed to all situations that it is likely to encounter during its life. The best prevention of behavior problems is to take the puppy to puppy classes during that time. During the socialization period, the puppy can already learn some commands. It should learn a biting inhibition, and should start to learn to fit into a social group.

Managing for Success: Problem prevention includes managing the puppy for success, i.e., arranging the environment so that the puppy cannot do the wrong thing, and automatically chooses to do the right thing (e.g., house training, chewing). If appropriate behaviors are successful from the puppy's point of view from the beginning, it will repeat these and not try other behaviors (and if we have set up the environment correctly, if it ever tries other behaviors, these are not successful). This includes puppy proofing the house and appropriate confinement and supervision.

Exercise: Exercise off the property will satisfy the dog's innate motivation to explore new things, help with exposure and desensitization to stimuli, and facilitate socialization. Exercise off the property also decreases arousal and reactivity, reduces anxiety and reduces the risk of owner-directed aggression. Vigorous exercise also reduces anxiety through its effect on stress hormones and serotonin.

Environmental enrichment: Interactive toys and games, food dispensing toys, rotating the toys so they maintain novelty, and appropriate play serve to enrich the environment and provide mental stimulation.

Obedience training: Obedience training has an enriching effect as well. Furthermore, humane obedience training (lure training, clicker training) provides for predictable, consistent and stress-free interaction, and an opportunity for the dog to act upon the environment with predictable outcome. If we are consistent in training, the dog has a lot of control over the situation (i.e., over the rewards). In clicker training, they literally can make us click. Furthermore, command-control over the dog can help diffuse critical situations by telling the dog what to do, i.e., providing the dog with an appropriate way to cope with a stressful situation.

In addition to usual commands, a puppy should learn a bite inhibition, food bowl safety, the "off" (or leave-it and drop-it) command, to walk on a leash, to be alone for some time, and to accept a crate. A puppy should also learn appropriate play.

Consistent Rules: I don't believe we have to dominate our dogs (I don't think our relationship with dogs is one of dominance and submissiveness, but that is open to debate). However, we have to control the contingencies on the dog's behavior. That means, we have to control consistently, which behaviors pay off for the dog (are rewarded) and which do not. The establishment and strict enforcement of rules are extremely important. If rules are not consistent, the dog can never figure them out, and cannot function within them to achieve success. This situation would be similar to you visiting with friends who play a card game that you don't know. They ask you to participate and explain you the rules. You go along with it and after some time think you have the winning hand, put your cards down and claim that you have won. Now of course, your friends add another rule, etc. After two or three times of this, you will become frustrated, angry (as a conflict behavior!) and distressed. This is how our dogs must feel if we have no rules or constantly change them. They may either compensate for this by developing survival behaviors that yield short-term predictable consequences (such as aggression, escape or inhibition), or develop learned helplessness (i.e., they learn that their behavior has no effect on what happens around them). Enforcing strict rules therefore has nothing to do with dominance, but a lot with giving the dog a chance to operate successfully in its environment and achieve predictable outcomes. Particularly a highly trainable dog, i.e., a dog that is keen on operating on his environment, is in a state of compromised welfare if a consistent rule structure is not maintained.

Managing the older dog

All the above also applies especially to older dogs that may suffer from declining cognitive function. Environmental enrichment, mental stimulation and teaching new behaviors (appropriate for the dog's age, health and physical ability) such as scent discrimination or searching for a hidden treat or toy, may help prevent cognitive decline. There are now classes offered for senior dogs. It is important to maintain older dogs' interest in participating in daily activities, social interactions, play and suitable exercise.

Andrew U. Luescher DVM PhD DACVB ECVBM-CA
Certified Applied Animal Behaviorist (ABS)
Director, Animal Behavior Clinic
Department of Veterinary Clinical Sciences
Purdue University
VCS, LYNN
625, Harrison Street
West Lafayette, IN 47904-2026, USA
E-mail : luescher@perdue.edu

Qualifications:

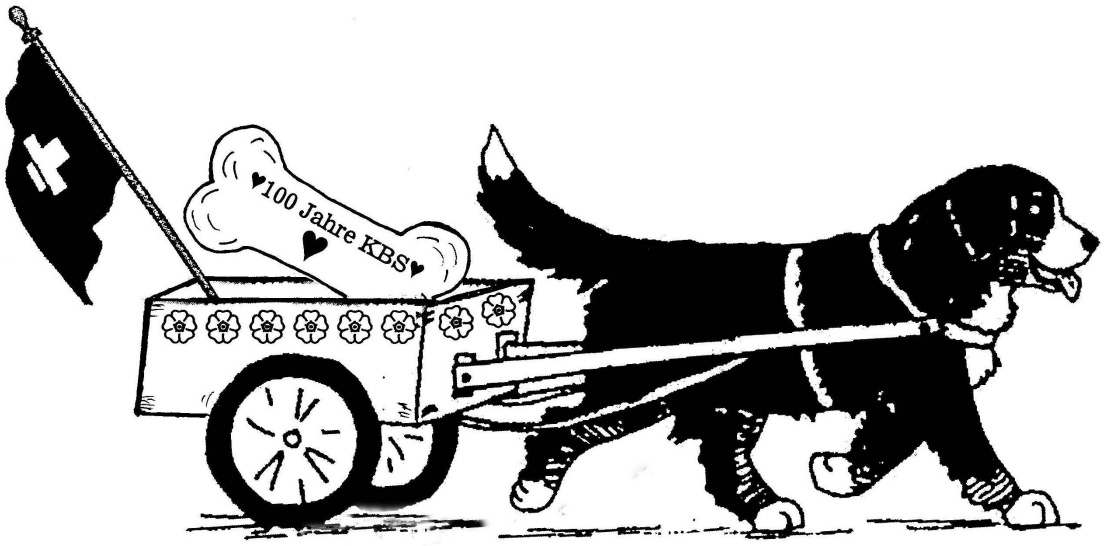
1975	Med. Vet. Prakt., Zurich, Switzerland
1979	Dr. med. vet., Zurich, Switzerland
1984	PhD (Animal Behavior) Guelph, Canada
1993	Certified Applied Animal Behaviorist (Animal Behavior Society)
1995	Diplomate, American College of Veterinary Behaviorists (ACVB)
2006	Diplomate, European College for Veterinary Behavior Medicine – Companion Animals

Professional Experience:

1982-1984	Research Associate, Federal Veterinary Office, Bern, Switzerland
1985-1997	Assistant Professor for Ethology, University of Guelph, Guelph, Ontario, Canada
1995-1997	Adjunct Faculty, animal behavior, Atlantic Veterinary College.
1997-	Associate Professor with tenure for Animal Behavior and Director of Animal Behavior Clinic, Purdue University, W. Lafayette, Indiana.

Research Interests:

Conflict behavior and compulsive disorder in various species
Canine aggression to household members
Psittacine behavior
Learning in dogs and training methods
Behavior Development



Practical Breeding Rules Adopted by the SSV to Improve Life Expectancy

Christel Fechler
Responsible for all breeding matters in the Swiss Mountain Dog Club (SSV) of Germany

Similar to many other breeding clubs, the SSV (Schweizer Sennenhund-Verein für Deutschland) began in the early ´90s to become very concerned about the very short average life expectancy of our Berners. Only occasionally we received information about Berners which had died. Generally such information was conveyed only by hearsay. We hardly ever received any written confirmation about the death of a Berner. In the past it was just accepted – although with great sadness – that a lot of dogs were dying at a much too young age. But now we ask for more details: What was the cause of death? Did the dogs undergo autopsy and was a pathological examination performed? We developed a questionnaire which was sent to all dog owners of whom we learned having lost their companions. The corresponding information was recorded, but at that time, we still had no effective program in place which would make this information available to the breeders and owners of Bernese Mountain Dogs. First steps were taken into the right direction.

We were also very concerned about how often certain stud dogs were being used. At that time all the appeals of our breeding warden were ignored, asking our breeders to take more responsibility and to make more use of stud dogs which had either no or very few offspring, as well as asking the owners of stud dogs to use them not so extensively but more selectively in making them available for mating. It was reality that individual stud dogs had up to 740 direct offspring alone in the SSV. Additionally, there might have been even more matings with the same stud dog in foreign countries.

After considerable discussion within the SSV as well as with the scientific advisory board of the VDH (Verein für das Deutsche Hundewesen, the German Kennel Club), new breeding guidelines were proposed and, in autumn 2004, approved and enacted by the SSV Committee.

Basically these breeding guidelines include the following regulations which we hope will lead to an increased life expectancy:

- Optimal use of the available breeding dogs, taking into consideration the age of their ancestors and siblings
- Comprehensive monitoring of the offspring
- Increasing the responsibility of stud dog and bitch owners

In this context, all breeders and owners of Bernese Mountain Dogs have been requested to ensure complete and open information with respect to age structure and the comprehensive monitoring of the offspring.

In more detail the SSV breeding guidelines and rules include:

a) To improve the age structure, Bernese Mountain Dogs which have been approved according to the Breeding and Selection Rules (“Körung”) of the SSV, and which can therefore be used in the breeding program, can only be considered for matings in which for at least 10 of the 14 ancestors (i.e. up to the great grandparents´ generation) of each of the partners in the planned mating, either some proof (not older than 12 months) can be presented that a dog is still alive, or a report of death. Decisions are made using the data on each dog before the mating. If the necessary data (life or death reports) are not available and can not be obtained before the actual mating, an application for permission of the mating can be forwarded by the breed warden to the Breeding Commission.

b) After having successfully completed the breeding evaluation test (Körung) and taking into consideration all the breeding requirements listed above, a stud dog is initially only allowed to sire a maximum of 21 litters which are to be registered in the SSV Studbook. Thereby, not more than 7 litters are allowed to be sired within a period of 12 months, counted from the day of the first live born litter. Stud dogs beyond their 8th year of age (=96 months) are not any longer restricted with regard to the number of matings.

c) For stud dogs younger than 8 years, which have already sired 21 litters registered in the Studbook of the SSV, an application can be directed to the Breeding Commission, which may allow further matings, taking into consideration the relevancy to the situation of the breeding population of the Bernese Mountain Dogs prevailing at the time the application is made. This

application is to be directed to the breed warden, explaining the reasons for the special application and also including the life/death reports of the ancestors, the litter siblings, as well as the offspring of the stud dog and the results of the offspring controls. “Offspring control “will be explained in detail in paragraph e).

d) Stud dogs, which were not yet 8 years old but which had already sired 18 or more litters registered in the studbook of the SSV before the new breeding regulations were enacted, were allowed to have 3 additional litters. All other breeding approved studs younger than 8 years were allowed to be used in the breeding program until they had 21 litters registered in the Stud Book of the SSV, including their SSV registered litters before the new breeding regulations were enacted. The stipulations in paragraph c) apply for possible further breeding use.

e) Brood bitches with offspring having reached the age of 18 months, are not allowed to be used again for breeding until at least 2 (in the case of a litter with only a single puppy, 1) of these offspring per litter (if possible 1 male and 1 female dog) have had HD and ED x-ray examinations comprising an official evaluation by the designated body of the SSV. This is what we mean by “offspring control” (Nachzuchtkontrolle). The Breeding Committee (represented by the breed warden responsible for the area) determines together with the breeder during the litter inspection, which of the puppies should be included in the offspring control. In case that one of the dogs chosen for the evaluation can not be presented for some reason (e.g. for health reasons), another puppy must be identified as “substitute offspring”. If the puppies chosen by the breed warden and the breeder at the litter inspection have not been evaluated at the appropriate age, the necessary offspring control can alternatively be conducted by an evaluation of 70% of the dogs from each litter.

The breeders are asked to keep the inbreeding coefficients as low as possible.

For the submission the living/death reports required by the new Breeding Plan, all members of our Club received special forms together with our Club Newsletter, the “SSV Kurier”. These forms can now also be downloaded from our Club Internet Homepage. The proof that a dog is still alive must either be certified by a registered veterinarian (e.g. at the annual vaccination) or by one of the SSV breed wardens. In addition, any participation of a dog in FCI shows, performance tests, offspring control, breeding evaluation tests – i.e. at official events – will be automatically recorded. Breeding notifications and litter reports for the sire and bitch involved, as well as vaccination certificates which are clearly assigned to a particular dog, are also accepted as proof that the dog is still alive.

Death reports may also be communicated informally as well as by use of the appropriate form. In any case either a pathology report should be attached or at least a statement must be included on whatever a veterinarian presumed to be the cause of death.

So far, the SSV is deliberately not insisting on a mandatory procedure according to which a pathology report must be submitted for each dog’s death, because we feel that in this way we will get a higher response rate of reports. Some breeders and dog owners are anyway unwilling to report a death, especially if the dog has died at a young age and of cancer, because they are afraid of damaging the reputation of their kennel. However, we certainly hope that in the future, in the interest of the breed, there will be a significant increase in the willingness of all owners and breeders to submit a pathology report on all dogs that have died or have been euthanized.

Just recently, in November 2006, to further improve the feedback rate of life/death reports, the SSV sent 847 questionnaires to the owners of the Bernese Mountain Dogs which had been born in the years 1997 to 1999. 458 of these forms (only 54%) have been returned. 305 of the dogs were still living at that time, and 153 were reported to have died. Unfortunately, most of these dogs had died – as expected – of cancer.

At this point in time we have received a total of about 5,100 death reports. All information on life or death of the dogs have been entered into a data base named “Dogbase” and are thus accessible to our breeders and club members. However, the cause of death has not been made public because only a very small proportion of our breeders have been honest and open enough to report all dogs from their kennels which have died at an early age with tumours. We must make sure that dishonesty does not bring any advantages for the breeders, and that openness and honesty as well as a high return rate of

reports is rewarded. For this reason, the up-to-date results of reports are made public once a year for dogs coming from individual kennels.

Generally, our experience is positive in dealing with our breeders regarding the new restrictive breeding regulations. Before we enacted the new regulations, we organized informational meetings in all of our 13 state groups in order to convince the breeders of the urgent necessity of our actions. At the same time special attention was given to elderly dogs at all SSV events and in the media.

I do not wish to hide the fact that there was a lot of resistance which occasionally persists today from a small percentage of our breeders. Some of these breeders – generally profit-oriented ones – have left the SSV in the meantime. However the persistent implementation of the breeding strategies has been accepted by most of the breeders and is also appreciated by the new puppy owners.

I think we have opened the eyes of many breeders to the problems of the unsatisfactory life expectancy of our Berners, and specifically to the fact that they have to take more interest in the age structure of their own dogs, as well as with that of the breeding partners they choose. Consequently, many of the breeders now accept advice from the breed wardens of the SSV regarding the breed planning. Unfortunately, I cannot deny the fact that some of our breeders still select mating combinations with only focusing on the phenotype conformation , all this in spite of repeated appeals from the SSV to also strongly consider health and life expectancy in future breeding.

The limited use of stud dogs has now been accepted by most of our stud dog owners. In fact, this is not a severe restriction, but rather a redistribution. Recently, very few stud dogs have reached the maximum allowed number of 7 litters a year, or a total of 21. Studs which have reached the age of 8 years are now being used more often for breeding. Unfortunately, there are not too many studs of this age which are still capable of siring litters. It is gratifying to see that the gene pool has already become much wider resulting from the limited use of individual dogs but using a significantly larger number of different studs.

The required information about the 10 of 14 ancestors is now available for all dogs registered or approved for breeding in the SSV. There are many dogs for which the necessary information is complete for all 14 ancestors and even further back in their ancestry. The information concerning litter siblings is, in some cases, still incomplete or not available. Most of the problems we face in data collection are with dogs from other countries. This is sometimes either very difficult or almost impossible to get. In spite of this problem, the use of studs from other countries has increased by more than a third. In the long run, a stronger cooperation between the different clubs and the members of the International Working Group is the way to move forward. We are so pleased that we have been able to collaborate and exchange data with several neighbouring clubs and other befriended clubs. On the positive side, we note that more and more data is being collected in other countries.

The provisional examination of our data by geneticists has shown that life expectancy is highly genetically determined. In the near future we hope to be able to define a breeding coefficient for life expectancy, which needs to be scientifically based. With this we hope to establish a new tool to reach higher life expectancy of our Berners, a tool to be used by both, the breeders and stud dog owners.

Because all these rules and measures were introduced only a short time ago, we certainly hope that they will lead to the success we are driving for in the future.

Christel Fechler Curriculum Vitae concerning canine activities

Owner of Bernese Mountain Dogs since 1962, joined the Swiss Mountain Dog Club (SSV) of Germany in the same year.
1974 Breed Warden of the SSV, since 1976 responsible for keeping the Stud Book and in this capacity member of the SSV committee. 1986 Special Conformation Judge for Swiss Mountain Dogs,
1991 Special Judge for Breeding Evaluation Tests, since 1999 Head Breed Warden of the SSV
Since 2002 Head of the Breeding Commission of the SSV.
In addition, President of the State Club in Nordrhein-Westfalen and Rheinland from 1975-1991,
1993-1998 Puppy and Breeder Referral Officer, since 1976 Breeder of Bernese Mountain Dogs.

Analysis of the genetic causes of Malignant Histiocytosis in Bernese Mountain Dogs

Dr. Catherine André

Benoît Hédan[1], Edouard Cadieu[2], Jérôme Abadie[3], Patrick Devauchelle[4], Heidi Parker[2], Clothilde de Britto[3], Elaine Ostrander[2], Francis Galibert[1] and Catherine André[1]

[1] Laboratoire de Génétique et Développement, UMR 6061 CNRS, Université de Rennes1, IFR140, Faculté de Médecine, 35043 Rennes Cedex, France
[2] NIH/NHGRI, 50 South Drive, MSC 8000, Building 50, Room 5339, Bethesda, MD 20892 – 8000, USA.
[3]Unité d'Anatomie Pathologique, UMR INRA-ENVN 703, Ecole Nationale Vétérinaire, BP 40706, 44300 Nantes, France
[4] Centre Anticancéreux Vétérinaire, Ecole Nationale Vétérinaire ENVA, 7, Av. General de Gaulle, 94704 Maisons Alfort.

Canine histiocytic proliferative disorders are heterogenous diseases that include reactive disorders such as cutaneous and systemic histiocytosis, and neoplasia such as cutaneous histiocytoma and localised and disseminated histiocytic sarcoma (malignant histiocytosis, MH). Their etiology and pathogenesis are unknown. They share many clinical and pathological features with histiocytosis in human beings, for which the underlying causes are still unknown.

Malignant histiocytosis is highly breed specific : its incidence in Bernese Mountain Dogs (BMD), Rottweillers and Retrievers is high, and MH represents nearly 25 % of the cause of death in the BMDs, the mean age being 6 years.

We have been working on MH in the BMD for 4 years, to better characterize the clinical forms, physiopathology, epidemiology by mean of a questionnaire sent to veterinarians referring MH cases.

Moreover, we undertook the search of the genetic causes of this dramatic disease. We collected blood samples and clinical data from BMDs in France and recently in other European countries and constructed a large BMD pedigree of 300 dogs, 100 of which are affected. All affected have an histopathogical report and are submitted to histological confirmation and when necessary a characterization through immunohistochemical analyses. We showed that the transmission mode of the disease is most probably oligogenic, involving a small number of genes. We also collected tumor and healthy tissue samples to analyze gene expression at the RNA level. In parallel, a set of 100 cases and controls from American BMDs have also been collected. Two complementary genetic approaches have been performed on the 2 sets of dogs (US and European) in order to localize the genes involved in MH in the BMD.

A total of 191 dogs from the "French pedigree" were analyzed though "genetic linkage analysis", with 260 microsatellite markers and the very first results, while still under investigation, point out several regions on 3 chromosomes as containing genes involved in the disease.
On the other hand, 55 validated MH case and 120 aged control dogs from the US collection were analyzed through an "association method" with a set of 500 microsatellite markers, results allowed the identification of several locus.
For both studies, statistical analyses are still running and interpretation of the data are continuing. Among the several chromosomal regions identified by both groups, at list four loci are common and under active investigation.

In conclusion, using two complementary genetic methods, and two sets of dogs, US and European BMD populations, allowed us to find concordant and very encouraging results. The identified loci are being examined into details to search for the genes or genome regions and their mutations involved in the disease. More analyses are needed to search for other loci. The future aims of this research are to develop genetic tests for diagnosis and early detection, as well as to transfer knowledge to human medical research for histiocytic disorders.

The authors warmly thank the numerous breeders and BMD owners and veterinarians for the collection of samples as well as AFBS (Association Francaise du Bouvier Suisse), CIAB, SIBB, Belgian Club and other European and American clubs and Margaret Baertschi and Didier Paineau for generously sharing their knowledge of the breed history.

Dr. Catherine André
Laboratoire de Génétique et Développement
UMR 6061 CNRS
Université de Rennes1, IFR140
Faculté de Médecine,
35043 Rennes Cedex, France
E-mail : catherine.andre@univ-rennes1.fr

Catherine André has graduated as a PhD in Molecular Genetics and Oncology at Paris University in 1992. She is working since 1995 at CNRS, University of Rennes in France,
on canine genomics and genetics. She manages the “Canine Genetics” group at CNRS which researches the genetic basis of inherited diseases in canines and humans.

Molecular Cytogenetics of MH in Bernese Mountain Dogs at North Carolina State University – a brief update

Dr. Matthew Breen

This contribution is presented by Dr. Catherine André

At the 2006 meeting in Como, Italy, Dr. Breen described how the main focus of his lab is to apply sophisticated molecular cytogenetic techniques, developed by his lab over the past 10 years, to identify regions of the canine genome associated recurrently with a variety of canine cancers, including histiocytic malignancies in the Berner. The genome of each cell of a dog is organized in chromosomes, sub-cellular features that Dr. Breen describes as nature's biological filing cabinets. When cells become cancerous it is common for the files (or genes) within these filing cabinets (or chromosome) to become disorganized. Identifying where these files (genes) move is a key step in learning how cancer begins and also very important in providing help in understanding how to halt cancer development.

With the availability of a high quality canine genome sequence, Dr. Breen described how knowledge of the chromosome changes in cancers now offered a means to pinpoint key genes involved in the cancers – the first step in the design of improved, targeted treatments. This study uses owner-submitted tumor biopsy samples recruited from Berners across the USA and also preserved specimens from tumors from all over the world. Dr. Breen described how accurate pathology information for each case was critical to making good progress and emphasized the power and importance of the ongoing multi-institutional collaboration between Dr. Ostander, Dr. Andre and his own lab.

Tessa Breen, Laboratory Manager of the NCSU Canine Genomics Laboratory, is the liaison between the owners/vets and the researchers at NCSU. She is also responsible for receiving ad processing all tumor samples submitted to the lab. Tessa described how Berner samples were recruited and processed to ensure that maximum benefit and potential information was obtained from each samples.

Since last year, Dr. Breen's lab has continued to recruit fresh tumors from Berners and now has almost 100 samples undergoing analysis. This extraordinary level of recruitment is testimony to the willing participation of the many Berner owners who have had the courage to submit tumor samples to the study. It is also indicative of the astonishing level of cancer, especially those of histiocytic origin that are affecting the Berner breed.

All cases recruited are evaluated by Dr. John Cullen, Professor of Pathology at NCSU and also President of the American College of Veterinary Pathologists. Dr. Cullen's assessment is that 69% of cases submitted to Dr. Breen thus far represent cases of malignant histiocytosis or histiocytic sarcoma, with approx. 10% of cases diagnosed as lymphoma, 10% hemangiosarcoma and the remaining 11% representing mainly a variety of soft tissue sarcomas.

Initial assessment of the cytogenetic profiles of many of these cases of MH/HS has revealed several regions in the canine genome that may contain significant genes involved in malignant histiocytosis in Berners. One of these regions is of particular importance, since it a chromosomal region corresponding to the same region that has been associated with malignant histiocytosis in Berners by the parallel study of Dr. Ostrander at NHGRI and Dr. Andre in Rennes. Whenever possible, Drs. Breen, Andre and Ostrander share the samples they recruit so that maximum benefit is obtained from each sample submitted. Some of the regions identified in Dr. Breen's study are quite large in genomic terms and so are now being studied in greater detail. This process will refine the data and so help to generate a shortlist of candidate genes of significance.

With a considerable amount of data generated from fresh case material, Dr. Breen is extending the study to include archival tumor samples – samples that were taken from Berners several years ago and stored as formalin fixed paraffin blocks – to determine if the same genetic changes have been present in the breed over a longer time frame.

Thanks to continued support from the Berner community and the AKC Canine Health Foundation, Dr. Breen's lab will continue to recruit cases for inclusion into this study for at least a further two years. In addition, with growing sample numbers, attention is also now being directed toward evaluation of the chromosome changes evident in lymphoma and hemangiosarcoma samples submitted from Berners.

Matthew Breen, PhD
Associate Professor of Genomics
College of Veterinary Medicine
North Carolina State University
4700 Hillsborough Street
CVM Research Building, Room 348
Raleigh, NC 2706, USA
E-mail: Matthew.Breen@ncsu.edu

1987	B.Sc. (Hons) Genetics, University of Liverpool, U.K.
1990	PhD Genetics/Cytogenetics, University of Liverpool, U.K.
1990 – 1992	PostDoc Molecular Cytogenetics, Medical Research Council, U.K.
1992 – 1996	Senior Research Officer, Head of DNA Group, Australian Equine Blood Typing Research Laboratory, University of Queensland, Brisbane, Australia
1996 – 2000	Cytogeneticist, Animal Health Trust, Newmarket, U.K.
2000 – 2002	Head of Molecular Cytogenetics, Animal Health Trust, Newmarket, U.K.
2002 – present	Associated Professor of Genomics, Dept of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh NC, USA

Investigation into the genetic background of malignant histiocytic tumors in Bernese Mountain Dogs

Dr. G. R. Rutteman

Gerard Rutteman[1], Heidi Parker[2], Elaine Ostrander[2], Catherine André[3], Matthew Breen[4], Guy Grinwis[5]

[1]Utrecht University Clinic of Companion Animals, POX 80.154, 3508 TD Utrecht, and Veterinary Specialist Centre De Wagenrenk, 6705 BN, Wageningen, The Netherlands
[2] NIH/NHGRI, 50 South Drive, MSC 8000, Building 50, Room 5351, Bethesda, MD 20892 – 8000, USA
[3] Laboratoire de Génétique et Développement, UMR 6061 CNRS, Université de Rennes1, IFR140, Faculté de Médecine, 35043 Rennes Cedex, France
[4]Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, North Carolina 27606, USA.
[5]Department of Pathology, Faculty of Veterinary Medicine, Utrecht University, The Netherlands

In the 1980s it was discovered that Bernese Mountain Dogs were sometimes affected with a wide-spread malignant histiocytic cancer, called malignant histiocytosis (MH) and a familial predisposition was shown. Shortly thereafter, collection of data regarding the disease began in the Netherlands (NL) and in other countries. Some animals, it was found, developed a localized form with high metastatic propensity that was often referred to as histiocytic sarcoma (HS). A few years ago an international consortium was established to collaborate in the scientific investigation of the genetic background of MH/HS. Our research involves analysis of the genome of affected animals and healthy veteran controls, through the use of high density genetic markers. NL is currently participating in a breed-wide association study, which does not depend upon family background.

Of 250 BMD that were reported to the UUCCA suspected to be affected with MH in the past 10 years, insufficient data leading to a positive diagnosis or lack of biological material excluded 100 from entry into the association study. In each of these cases, either no blood had been collected, or the diagnosis had been based on clinical signs and radiographic examination without examination of the tumor tissue. Of those dogs where cytological and/or histological examination was possible, 15 did not have cancer, and 35 dogs with masses in bone or near the joint (HS), or in thorax or abdomen (sometimes central nervous system) had a variety of other cancers, such as multiple myeloma, osteosarcoma, bronchial carcinoma, liver or ovarian carcinoma and malignant lymphoma, not MH. Of these tumor types, the latter (malignant lymphoma) is most often differentiated early on because access is easy and immuno-histochemistry can identify the true histogenetic type. Of the 80 unaffected control dogs that donated blood at > 8 years of age, subsequent follow-up found that 8 died of unknown causes, 10 died of tumor or immune disease with suspicion of HS/MH, but without verification, and 12 developed either HS/MH (with transport into the MH/HS group) or another malignant tumor that necessitated exclusion from the control group.

From this experience we found that, for an effective genetic study, proper identification of the tumor type by laboratory analysis, with central revision, and constant follow up regarding health status is essential.

Dr. Ostrander's group at the NIH has recently completed a scan using 500 microsatellite markers spanning the entire canine genome in 350 Bernese mountain dogs, both affected and unaffected, coming from the USA, France, NL and several other European countries. Focusing on a subset of 55 Bernese diagnosed before the age of 8 and 125 unaffected Bernese over the age of 10, we have identified four regions of the genome that are likely to contain MH susceptibility genes. The combination of our findings and the results obtained by Dr. André et al. at the University of Rennes based on an extended family of Bernese highlights two highly significant regions that are currently under investigation. We are working on fine mapping in these regions in order to find the specific genes that are responsible for the disease while at the same time applying new SNP genotyping technologies to our population samples in order to verify our findings and identify additional loci.

Furthermore, tumors that are rapidly frozen (or 3 mm particles put in a vials containing a special solution: RNA later) are being examined by Dr. Breen et al. at North Carolina State University using the high-tech cytogenetic technique, comparative genomic hybridization (CGH). With dozens of tumors thus

analyzed, common patterns, such as genomic amplification or translocation or deletion of specific chromosomal areas, can be deciphered. Identification of such regions may help both in the localization of important genes and also in discovering the mechanisms behind cancer development. From NL a shipment of about 35 samples will be shipped to NCSU next month for extension of the CGH analysis.

In addition, combination of data from the above NL cases with data from archives from collaborating laboratories from the past 15 years, will be used to assemble a group of > 300 MH/HS cases, to be examined for possible differences of disease manifestation (MH versus HS) or age of occurrence between families.

In the coming years, the collaborating scientists will call on societies, breeders and owners to continue to inform other BMD owners to report dogs suspected of developing malignancies and to provide the study with blood and tissue. Great urgency also exists with respect to the entry of veteran BMD into the control group. Unaffected dogs can enter the study as controls by providing data on their health together with a blood sample.

Acknowledgement:

The authors are grateful for the help from owners and breeders of BMD and the national BMD societies, in the collection of data and material. Also, they wish to thank veterinarians from many European countries and the USA, who - often without putting in a charge - enabled collection of blood and tissues.

Gerard R. Rutteman, PhD
Utrecht University Clinic of Companion Animals
POX 80.154
3508 TD Utrecht and
Veterinary Specialist Centre De Wagenrenk
6705 BN, Wageningen, The Netherlands
E-mail : gerrjeak@tele2.nl

1978: DVM-degree, University of Utrecht, “cum laude”
From 1980 employed at the Utrecht University Clinic of Companion Animals;
first as scientific investigator (“fellow”), from 1986 till present as assistant Professor.
In 1988 PhD degree (thesis: Investigations on the pathogenesis of canine mammary tumors) at University of Utrecht.
1991: Specialist Internal Medicine of Companion Animals (Royal Dutch Society of Veterinary Medicine).
1997: Diplomate of the European College of Veterinary Internat Medicine (ECVIM-CA)
2005: Specialist Oncology (ECVIM-CA)
From 1996 onward part-time employed at the Veterinary Specialist Center “De Wagenrenk”