

**Benoit Hedan- CNRS- Rennes- France, June 2007**

**REWARDED BY SIBB Società Italiana Bovaro del Bernese WITH THE :  
2007 AVA Alberto Vittone Award € 10.000**

The AVA application is intended to Dr. Benoit Hédan, for accomplished research work on MH in the Bernese Mountain Dogs (BMD) and especially for future, to continue the study a few months before leaving for a post doctoral position in Drs. Matthew and Tessa Breen laboratory, (NCU, Raleigh, US).

### **The search of the genetic causes of Malignant Histiocytosis in the Bernese Mountain Dog.**

#### **Purpose of the research**

Malignant Histiocytosis (MH), severely affect Bernese Mountain Dogs (BMD), indeed at present the frequency of the disease can be estimated to 20-25 %. Males and females are equally affected and the mean age at diagnosis is 6 years. The diagnosis is often very late, the mean survival time after diagnosis is one and a half month. Up to now, there is no biochemical test allowing the detection or prediction of the disease. Generally, dogs already produced puppies before their MH has been diagnosed, since the onset can be late in a dog's life. Such a disease, with a high frequency in the breed, with a late onset and being fatal, induced a dramatic decrease of the life span of the Bernese Mountain dogs. Moreover, the only certitude diagnosis is given by histopathology, which is unfortunately not systematically done, thus the cause of the death of many dogs is not known. The condition is clearly familial, but until now, the genetic causes are unknown. Due to breeding practices of the last decades, using high inbreeding and few champions for reproduction– some of which being affected or carriers-, the situation now is very concerning, since it appears that MH affect any BMD, in all countries, Europe and US, with high frequencies. This is a case where genetic testing would be highly desirable.

Histiocytic disorders are also diagnosed in human, Langerhans Cell Histiocytosis (LCH) is the most represented form. While very rare (less than 50 cases in France), some forms are particularly aggressive, being treated by chemotherapies. These forms resemble MH

in the BMDs, while the more “classical” forms of LCH would mostly resemble the canine reactive histiocytosis. Research in human are very scarce and presently, the BMD presenting a spontaneous form of this disease could greatly help researches in the medical field. Veterinary and human medicine could mutually benefit of one and other, for genetic research, treatments...

Four years ago, and in collaboration with the laboratory of Dr. Elaine Ostrander, NIH, Bethesda, we focused on the genetics of canine cancer and especially MH in the BMD. This subject was given to Benoit Hedan, a veterinarian willing to perform genetic research. The work performed by Benoit Hedan on MH, for the last 4 years constitutes the main part of his PhD and two publications on the subject are in preparation, in international journals. B. Hedan’s PhD focused on malignant histiocytosis, a canine histiocytic proliferative disorder. The aim of this study is to search for the genes involved in this disease.

Different genetic approaches to identify genes are undertaken in 4 laboratories : Dr. Mathew Breen, Dr. Elaine Ostrander, Dr. S. London in the US and Dr. Catherine André, in the CNRS, Rennes, France. Mathew Breen subject deals on cytogenetics and large genomic approaches to identify chromosomal alterations. Dr. Elaine Ostrander uses a genetic method, by the collection of cases (affected dogs) and controls (healthy dogs) and Dr. Catherine André and Benoit Hédan performed a genetic linkage study, using a large BMD family. Finally, Dr. S. London deals with expression data in MH tissues. Very soon after the beginning of each project, we undertook a general large collaboration between the 3 labs. In Rennes, we developed a network with veterinarians, histology laboratories and BMD breeders to collect 600 BMD samples, owing to the French breed club, many breeders and owners and the many vets who sampled for us, as well as more recently, several other European BMD clubs . Most of those dogs, 200 of which had MH, could be connected in a large pedigree. Out of it, an informative family of 191 BMDs (with 74 MH affected dogs) could be constituted, by collecting samples, and clinical and genealogical data. Analysing the mode of inheritance of MH in this large family led to the hypothesis that the disease was transmitted through an oligogenic mode, ie that a little number of genes were necessary for the expression of MH. In the course of the sample collection, we made also an epidemiologic and clinical characterization of the MH in the BMD, by analysing clinical and genealogical data of 60 MH affected dogs from the pedigree; an article on this work is in preparation. Year 2006 was devoted to the genetic analyses of these 191 dogs with 400 genomic markers located on all the

canine chromosomes. These experiments (long and expensive) allowed us to identify five chromosomal loci ie five regions in the genome, thought to contain the searched genes.

The next steps will consist in precise analysis of each region and to search the genes in the loci. This work is collaborative between Dr. Elaine Ostrander and our group, and chromosomes to analyse are shared between us, to optimize the work and chances of success. This is a difficult but very exciting task since the loci are large and contain hundreds of genes. The aim of this research is double :

For veterinary medicine : The identification of these genes will allow to develop genetic tests for diagnosis and screening, it will allow better treatments and follow-up for malignant histiocytosis

For human medicine ; The identification of these genes will allow to check in human if the identified canine gene is or not involved in a series of patients affected by the corresponding diseases.

For fundamental research, It will of course help to understand better the mechanisms of histiocytic pathways and regulations.

### **Scientific program**

The present project now aims at identifying the genes responsible for MH located in the recently identified chromosomal regions. Since the sampling still continues and is even accelerated, Benoit Hedan selected 130 novel BMDs samples closely related to the first family of 191 dogs. Analyzing these dogs within the identified loci will hopefully reduce the interval on the chromosome in which we'll track the genes. Thanks to our operational network, we collect an average of 5 BMDs per week with 1 or 2 MH affected dogs. The used statistical method (non parametric genetic linkage analysis) will hopefully help to reduce the size of the identified loci, to identify easier the causative gene. During the previous study , we also collected 30<sup>th</sup> tumor samples, to analyse the expression of genes. This part will greatly help to point out up or down regulated genes in MH versus normal dogs and will be part of the project.

### **Length of the study**

The experimental part of the study will take 3 months (experiments, statistical analyses, interpretation of data). We hope that loci will be sufficiently reduced so that evident candidate

genes can be selected to be sequenced or to specifically analyse the expression to find the causal gene(s).

Catherine André and Benoit Hedan greatly thank AKC-CHF American Kennel Club – Canine health foundation- and the International Working group, who invited us to present our research work, as well as Pat Long from the Berner Guard foundation, for the announcement of our research work.

## **HEDAN Benoit**

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### **Curriculum vitae**

#### **Education**

- September 2003 - June 2007 : **PhD of genetics – Research of genes involved in canine genetic diseases, as models for human diseases : malignant histiocytosis of the Bernese Mountain dog and the Merle coat color** – Supervisor : Dr C. ANDRE, UMR6061 Génétique et Développement de l'université Rennes1/CNRS (FRANCE).
- 2003 : **Master « Animals and human biology » - Research of genes involved in genetic cardiopathy** - Supervisor J.J. Schott (INSERM U533, Institut du thorax, Nantes FRANCE).  
**Diploma of Animal experimentation**, first Level – Ecole Vétérinaire de Nantes (FRANCE).  
**Diploma of Experimental surgery** – Ecole Vétérinaire de Nantes (FRANCE).
- 1998-2003 : **Veterinary school and Diploma of Veterinary Medicine (DVM)** – Ecole Vétérinaire de Nantes (FRANCE).
- 1997 : **Baccalauréat scientifique (equivalent to A Level)** specialized in Mathematics.

#### **Work experience :**

- 2003-2006 : **Supply veterinarian.** Veterinary clinic of St Erblon (Rennes, FRANCE).

#### **Skills :**

- **Molecular Biology**
- **Bioinformatics, statistics**

➤ **Veterinary**

**Responsabilities :**

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- Supervision of junior and graduate students, or visitors
- Setting up networks for sample collection with breeders, veterinarians, histology labs
- Participation to working groups : « genetics for veterinary », « coat color genetic research », « human/canine histiocytosis disorders »

**LANGUAGES :**

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- **English and spanish** : written and spoken

**PUBLICATIONS:**

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- Hedan B., Corre S., Hitte C., Dreano S., Vilboux T., Derrien T., Denis B., Galibert F., Galibert M.D., Andre C. **Coat colour in dogs: identification of the Merle locus in the Australian shepherd breed.** *BMC Vet Res*, 2 :9. 27 February 2006.
- Vilboux T., Chaudieu G., Hitte C., Dréano S., Janin P., Derrien T., Delattre D., Hedan B., Queney G., Bourgain C. Galibert F., Thomas A. and André C. **Identification of the locus for Progressive Retinal Atrophy in the Border collie dog: a novel locus for human X-linked Retinitis Pigmentosa.** *In prep*
- Hédan B. et al. **Genetics, epidemiology and clinicopathology of malignant histiocytosis in Bernese Mountain Dogs.** *In prep*

Hédan B., Cadieu E., ..., Andre C., Ostrander E. Identification of loci in malignant histiocytosis : a canine model for human histiocytic disorders. . *In prep*

**POSTERS :**

- Andre C., Kerns J., Hedan B., Comstock K., Devauchelle P., Sutter N.B., Vilboux T., Galibert F., Ostrander E.A. **Mapping of gene for Malignant Histiocytosis (HM) in the Bernese Mountain Dog (BMD).** *2<sup>nd</sup> International Conference : Advances in Canine and Feline Genomics.* Utrecht, Netherlands, 14-16 october 2004.
- Abadie J., Hedan B., Vilboux T., Devauchelle P., Galibert F., Ostrander E., Cherel Y., Andre C. **Reactive and neoplastic histiocytosis of bernese mountain dogs: towards the identification of predisposing genes.** *Proceedings of the 23th Meeting of the European Society of Veterinary Pathology*, p13. Naples, Italy, 7-10 septembre 2005.
- Hedan B., Corre S., Dreano S., Vilboux T., Denis B., Galibert F., Galibert M.D., Andre C. **Coat color in dogs: a powerful model for mammalian pigmentation.** *International Pigment Cell Conference.* Reston, USA, 18-22 September 2005.
- Andre C., Hedan B., Vilboux T., Derrien T., Hitte C., Galibert F. **Le Chien : apports de ce nouveau modèle en génétique humaine.** *Assises de Génétique Humaine et Médicale.* Montpellier, France, 26-28 janvier 2006.
- Cadieu E., Hedan B., Parker H.G., Rutteman G., Bafoe-Bonnie A., Devauchelle P., Mosher D., Hitte C., Abadie J., Deshmukh, Galibert F., Breen M., Andre C. and Ostrander E.A. **Genetics of malignant histiocytosis in the bernese mountain dog.** *Genes, Dogs & Cancer : 4th International Canine Cancer Conference.* Chicago, USA, 14-17 september 2006.

**Research communications:**

- Hedan B. and André C. **Recherche du gène responsable du phénotype « merle » chez le chien (anomalies de la pigmentation et du développement).** *Groupe de travail sur la couleur des mammifères.* Limoges, France, 20 juin 2005.
- André C., Hedan B., Vilboux T., Devauchelle P., Abadie J., Ostrander E.A. and Galibert F. **Histiocytosis of bernese mountain dogs : towards the identification of predisposing genes.**

*Symposium on Swiss dogs health*. Salzburg, Austria, 24-25 novembre 2005.

- Devauchelle P., Andre C., Hedan B., Abadie J., Ostrander E.A. and Galibert F. **Les tumeurs histiocytaires : données clinique, anatomo-pathologique et recherche des causes génétiques**. *AFVAC*. Toulouse, France, 1-4 décembre 2005.
- de Brito C., Hedan B., Devauchelle P., Ostrander E., Galibert F., André C. and Abadie J. **Epidemiological and clinicopathological features of malignant histiocytosis in the bernese mountain dogs : a retrospective study on 50 cases**. *ECVIM-CA Congress*. Amsterdam, Netherlands, 2006.

#### **Informative and popular communications:**

- Bernese mountain dog meeting. Nevers April 2003 and 2005 and 2006
- **Recherches des causes génétiques de l'histiocytose maligne du Bouviers Bernois**. French bernese mountain dog review, 44, 2004.
- André C. and Hedan B. **Etude génétique de l'histiocytose maligne du bouvier bernois**. *Pratique Vet Anim Comp*, 14. march 2005.
- André C. and Hedan B. **Coup de projecteur sur l'histiocytose**. *Pratique Vet Anim Comp*, 25. march 2006.

#### **Lessons**

- **The dog a powerful model for genetic studies**. Agricultural Engineering school. Rennes, 4 april 2006.