IWG 2018 for Bernese Mountain Dogs:
Do tests make the difference ?

Canine Histiocytic Sarcoma in BMDs:
Genetic progresses and update on
- the HS genetic risk test, and
- the mating prediction test

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HISTIOCYTIC SARCOMA IN BMDs

Research : A long time collaboration with international teams
Development of the Genetic Risk Test : a long time collaboration

Benoit Hédan, CNRS and Caroline Dufaure, Antagene

AFBS

Chantal Orellou
Joelle Bardet,
Christine Malherbe

French AFBS and breeders
European Clubs and breeders,
American Berner garde, BMD Club,
International Working Group …

antagene

ANTAGENE: Anne Thomas
Caroline Dufaure de Citres
Guillaume Queney

Histiocytic Sarcoma in Humans

In humans

- Rare and mis-diagnosed aggressive tumor
  (1-5 cases per year in France)

- derived from monocytes/macrophage
  histiocytic markers : CD163, CD68, Lys…

- localized or disseminated (lymphe nodes, spleen, skin)

- limited response to chemotherapy and high mortality after several lines of treatments.

- no consensus on prognostic factors and treatments *(Takahashi et al. 2013)*

Photo credit Jean Donadieu

-> Very similar disease as HS in Bernese Mountains dogs
→ Fight this cancer in dogs and humans

Histiocytic Sarcoma in dogs

In dogs

- rare in the dog species, but frequent in breeds: BMD, Rottweilers, Retrievers
  Familial transmission: oligogenic mode (*Abadie, Hedan et al 2009*)

- highly aggressive cancer: localized or disseminated
  (lymphe nodes, spleen, skin, lung, joint…)

- 20-25% of BMD are affected by this tumor type

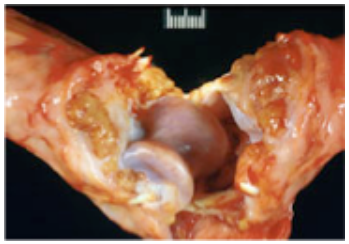
- expression of histiocytic markers : CD204/class A macrophage scavenger receptor+ (*Kato et al 2013)* : a good diagnostic marker

- aggressive disease with a poor prognosis : mean survival time :
  49 days for BMD, 123 days for FCR (*Abadie, Hedan et al 2009*)

Histiocytic Sarcoma: breed specificities

Different clinical forms

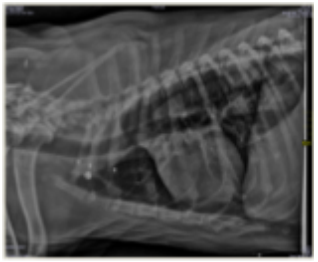
Localized forms :
Skin/peri-articular



www.histiocytosis.ucdavis.edu/sarcoma.html

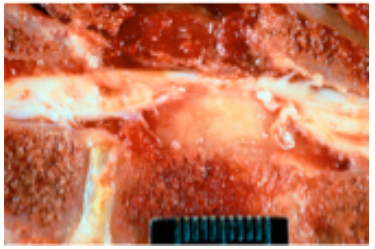
Retrievers

Disseminated forms :



BMD

Subdural forms :
Cerebrum/spinal cord

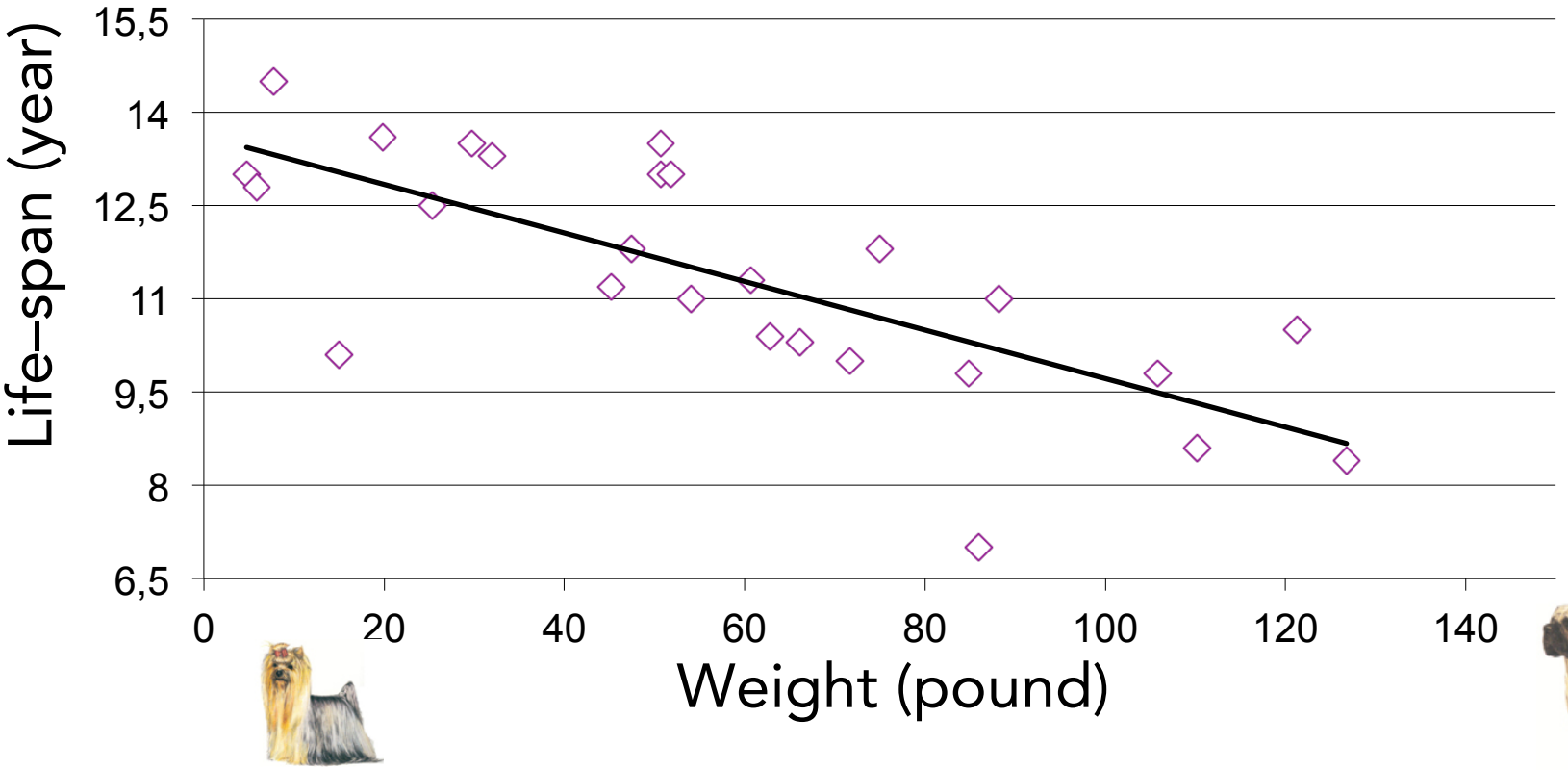


www.histiocytosis.ucdavis.edu/sarcoma.html

Pembroke Welsh Corgi

Bernese Mountain Dogs : lifespan

- Last 30 years: dramatic impact of cancers on the lifespan !



Bernese Mountain Dogs : lifespan

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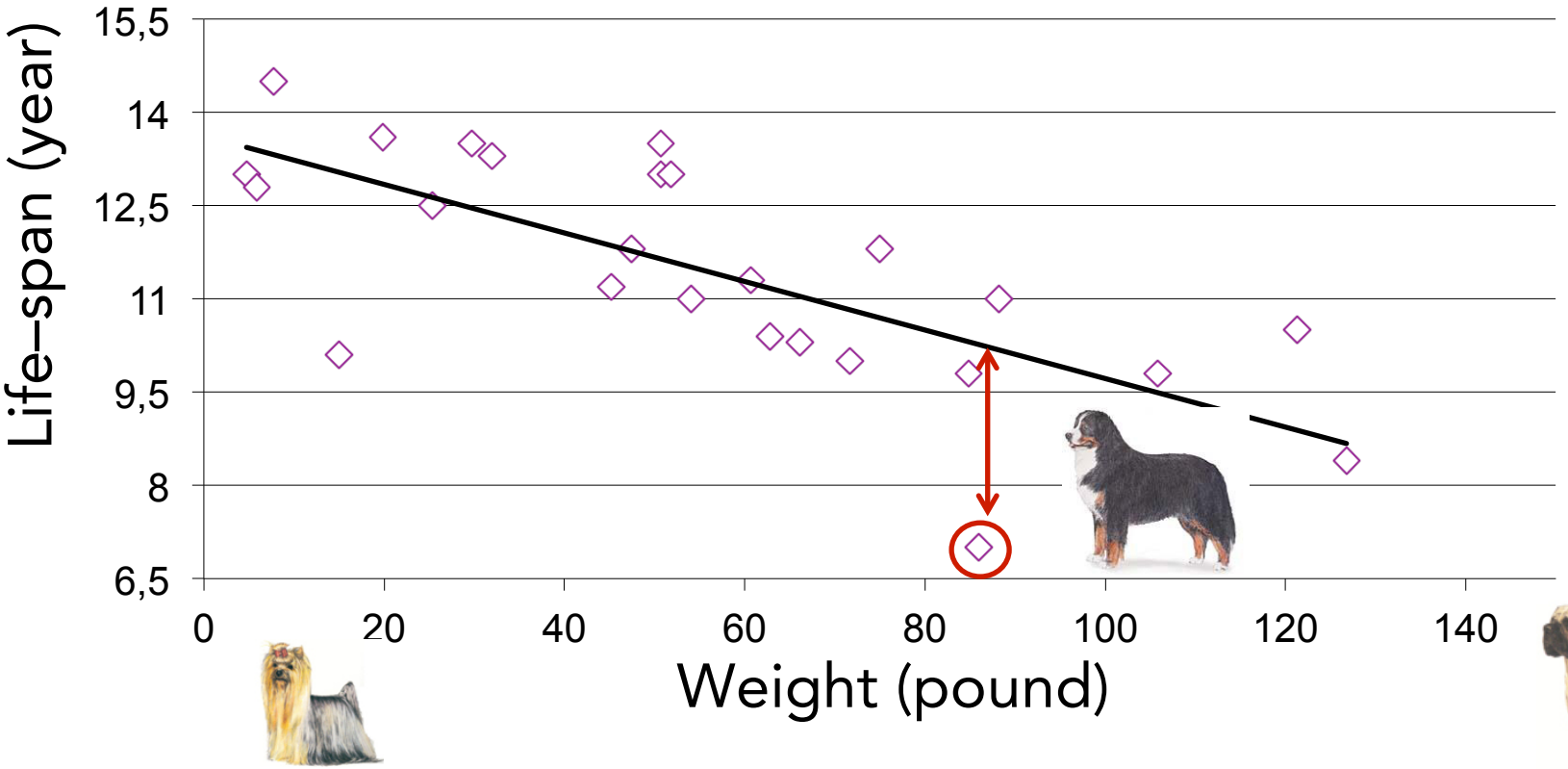
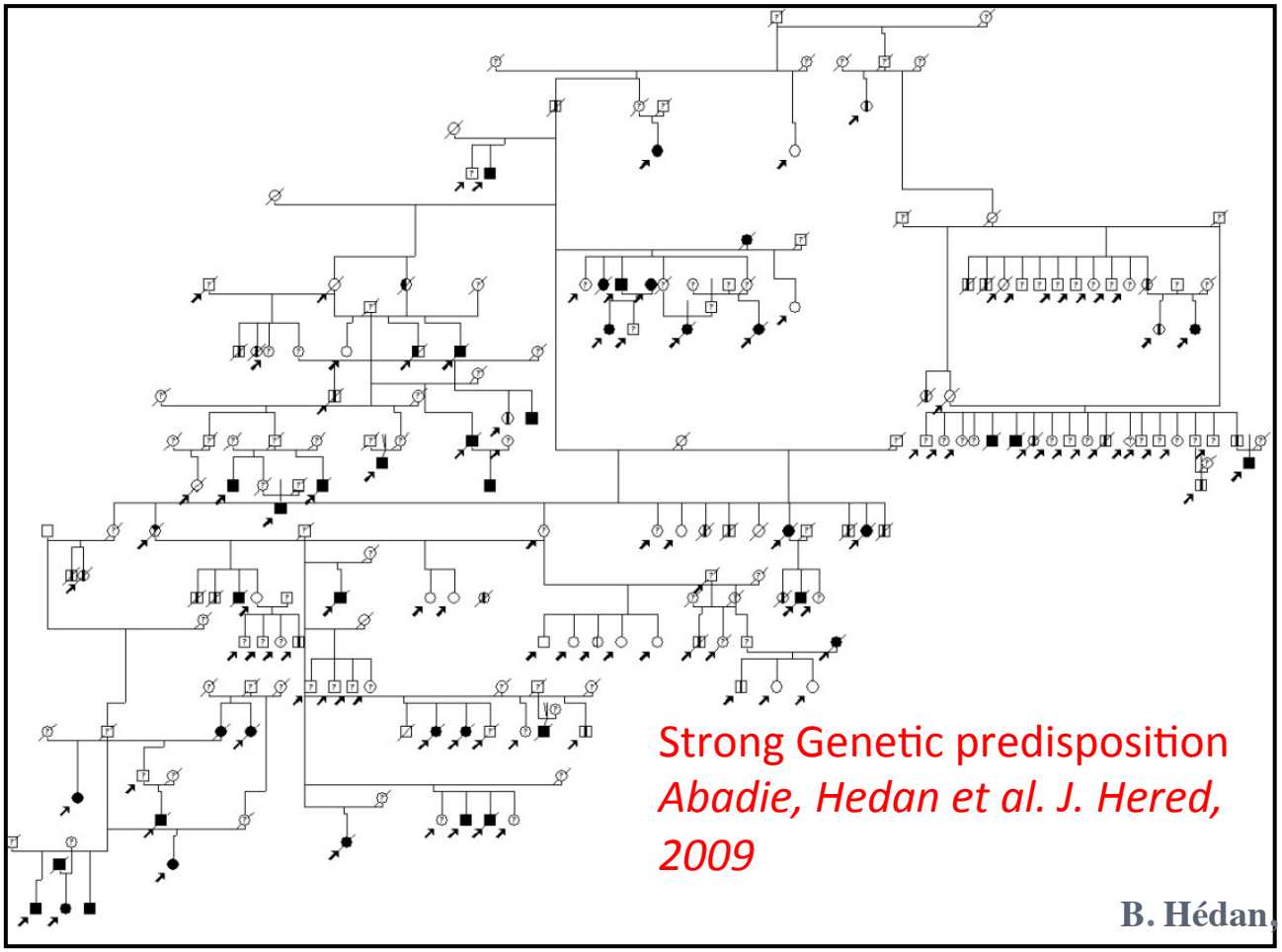


Bernese Mountain Dogs : genetic transmission of cancers

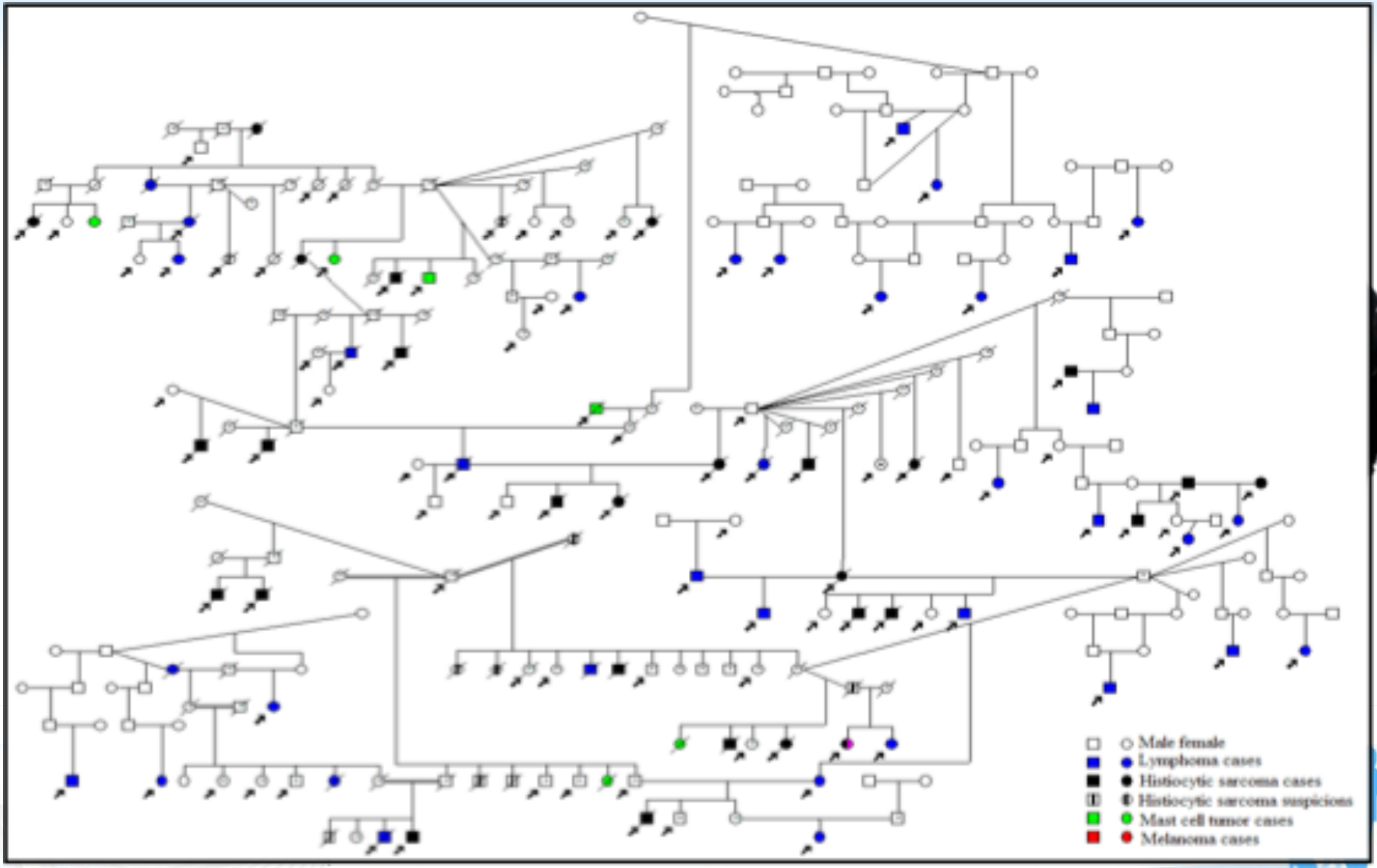
Since 2002: sample collection in the Cani-DNA French biobank

- > 4000 blood samples and > 400 tissue samples (paired tumour/healthy)
- > 450 HS cases with a pathology report

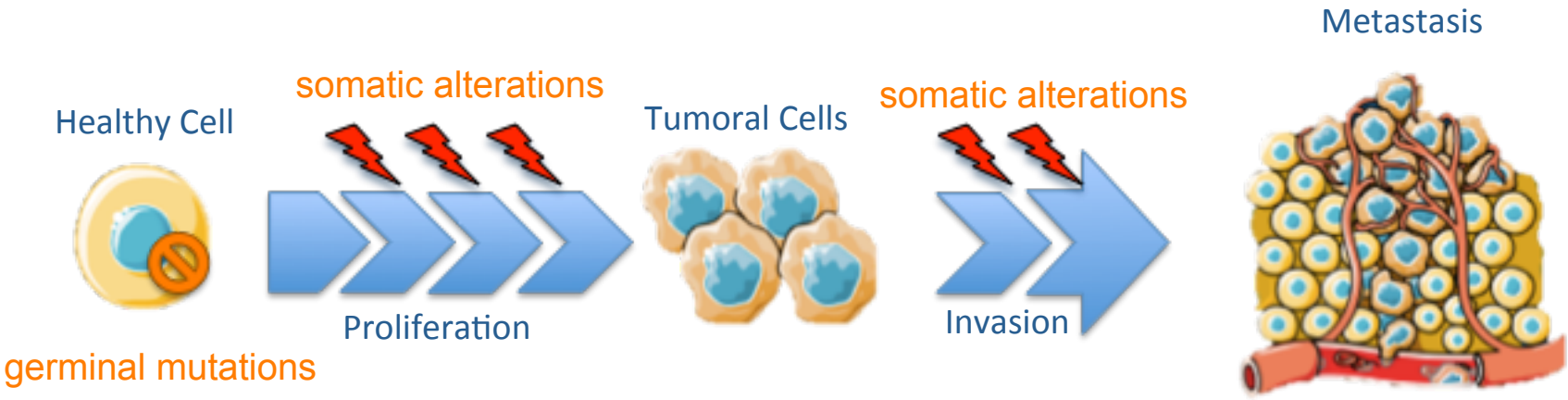


Strong Genetic predisposition
Abadie, Hedan et al. J. Hered, 2009

Bernese Mountain Dogs : genetic transmission of cancers



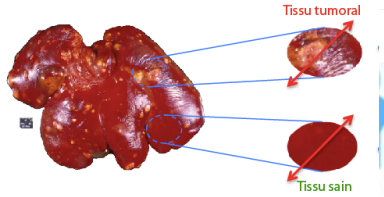
A long term goal : 2002 – 2018 !!!
Identify genetic mechanisms involved in HS
Sample collection since 2002



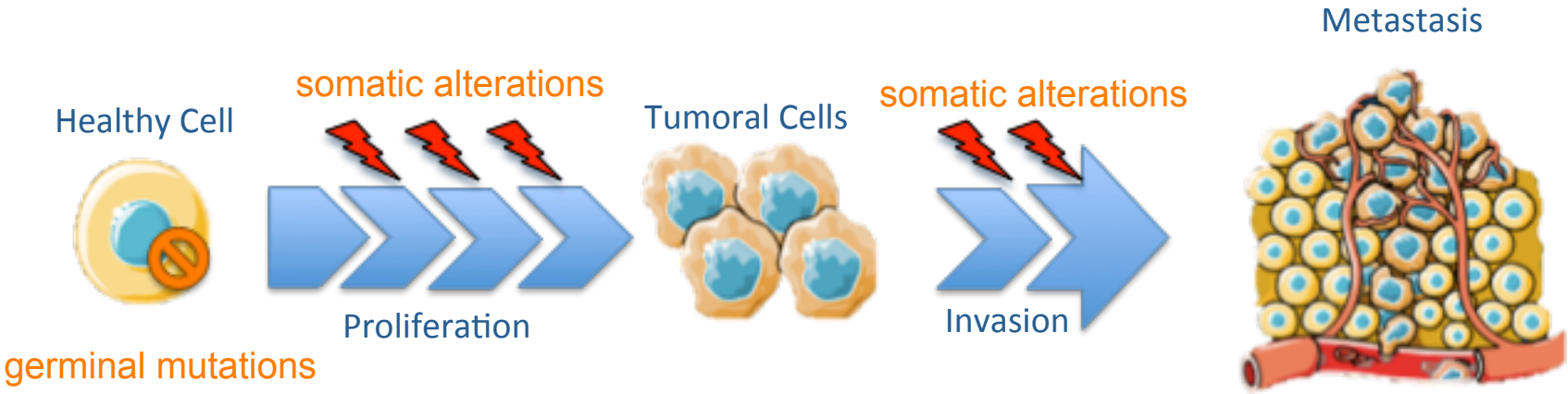
- Identify predisposing genes and risk alleles (germinal mutations)
  -> Blood collection

- Identify recurrent somatic alterations associated with tumor progression

-> Tissue collection



A long term collaboration : Identify genetic mechanisms involved in HS Collaborative work since 2006



- **Identify predisposing genes and risk alleles**

E. Ostrander's lab, *Shearin et al. 2012*
The Cancer genetics branch NIH (Bethesda, USA)

- **Identify somatic alterations** associated with tumor progression

M. Breen's lab, *Hedan et al. 2011*
NCSU (Raleigh, USA)

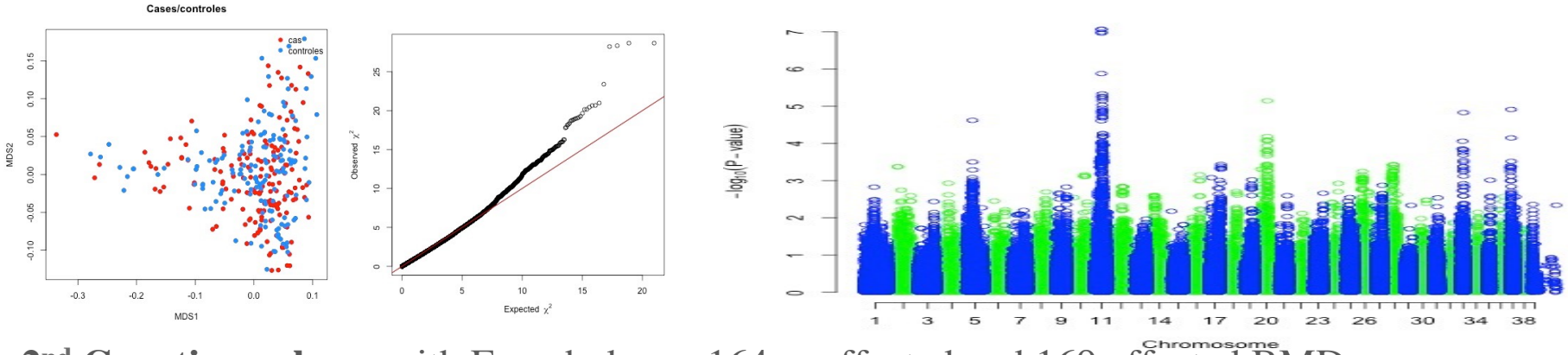
Searching predisposing genes:

➢ **Continue sample collection through Cani-DNA (dog.genetics.genouest.org)**

- > 4000 blood samples and > 400 tissue samples

➢ **In coll. with E. Ostrander's lab (NIH, Bethesda) (2006-2013): analysis of 500 dogs**

1st Genetic analyses (GWAS) with 232 unaffected and 244 affected BMDs (American/Europea
-> Identification of main chromosomal loci : CFA11, CFA14 *(Shearin, Hedan et al. 2012)*



2nd Genetic analyses with French dogs: 164 unaffected and 169 affected BMDs
→ Confirm and refine the localisation on chromosomes
→ Identification of MARKERS with Risk or Protective alleles

Research data can be useful for breeders
→ Development of a genetic risk test

- **We had identified main chromosomal regions involved in HS predisposition**
- **Cancer = Multifactorial disease -> involves genetic and environmental factors**

→ Still need research to understand the initiation and progression processes, to diagnose, prevent and treat …..

BUT, data produced by research can be useful for breeders to help selection

In 2012, in collaboration with Antagene and AFBS:

→ development of a **genetic risk test**

- Based on the previous genetic data: selection of 140 MARKERS associated with HS
- Genetic analysis of 1081 French BMDs (256 affected dogs + 165 unaffected dogs)

-> Selection of **9 SNPs significantly associated with the risk to develop HS**

Collaboration CNRS / Antagene / AFBS
Development of a genetic risk test for breeders

2003 — Identification of predisposition — 2011 — Pretest developpement — Genetic Risk Test — 2018

>1000 BMD

Technical developpements

Pilot study with 2 breeders : > 100 BMDs

2012

Tests on other breeds

2013

Validation steps:

European population (144 dogs)

American population (91 dogs)

2014

Numerous meetings with the Clubs, breeding commission and IWG (France, Italy, Switzerland, Belgium, Netherland Finland, Sweeden, Norway, Germany, …)

2016 Development of HSIMS

2019 Improvement of the HS test

Development of a genetic risk test for breeders
Selection of disciminent markers

In 2012 , in collaboration with Antagene, we developped a **genetic risk test**

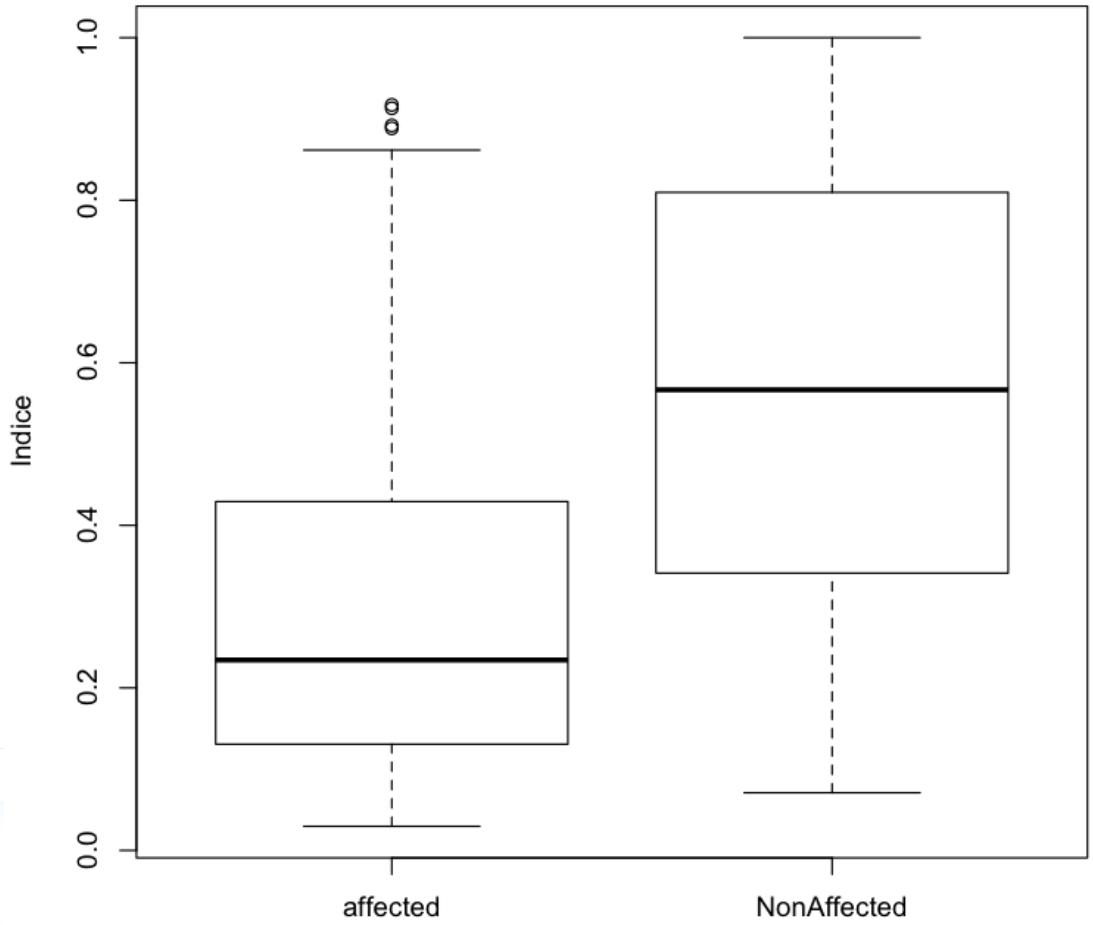
- Based on our genetic data: selection of 140 SNPs associated with HS
- Genotyping of 1081 French BMDs (256 affected dogs + 165 unaffected dogs)
  -> selection of the 9 Markers significantly associated to the risk to develop HS

Dog caryotype:
38 chromosomes + X,Y

- We used a statistical test to estimate the risk to be unaffected / affected depending on the alleles of the 9 markers and calculated an index, A, B, C

Development of a genetic risk test for breeders
Definition of an Index

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

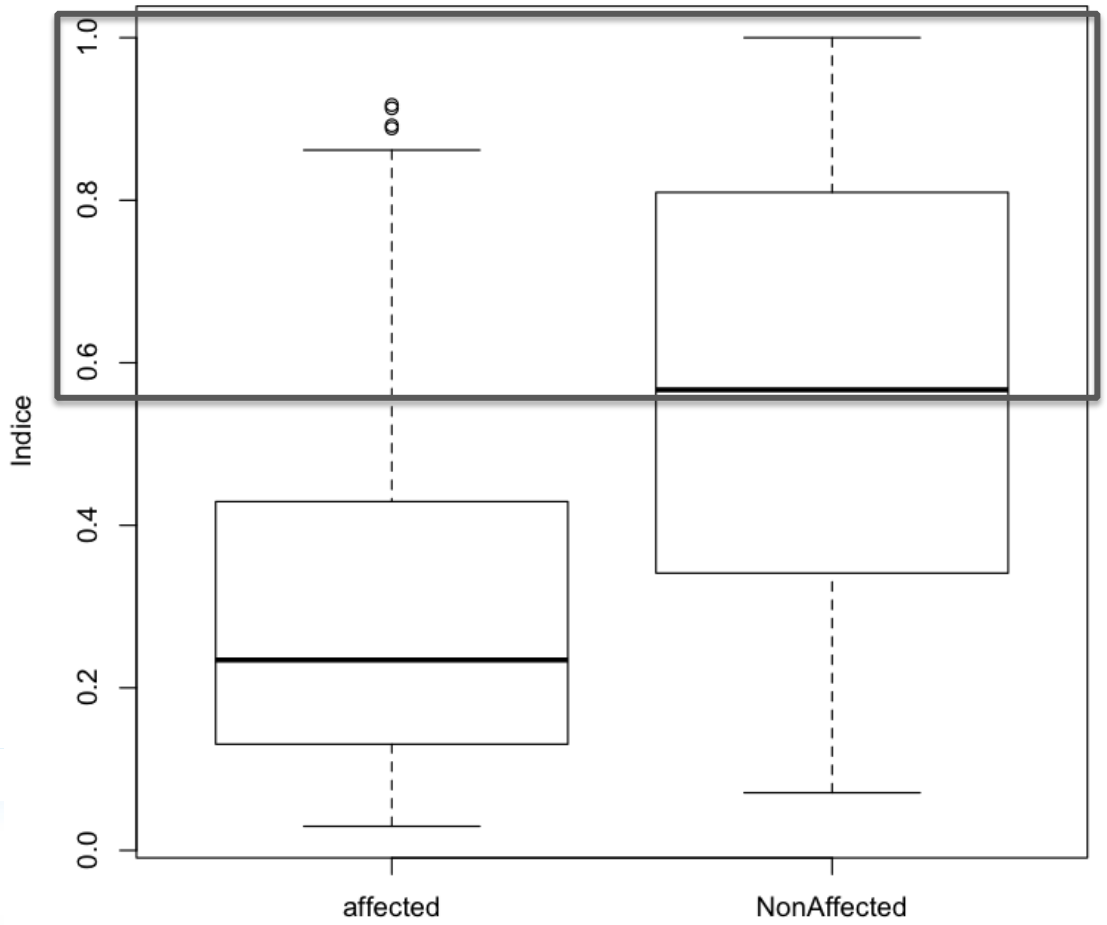


p-value : 1.754 10-16 (Mann Whitney test)

Development of a genetic risk test for breeders
Definition of an Index

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

INDEX A



47 % of healthy
10% of affected

p-value : 1.754 10-16 (Mann Whitney test)

Development of a genetic risk test for breeders
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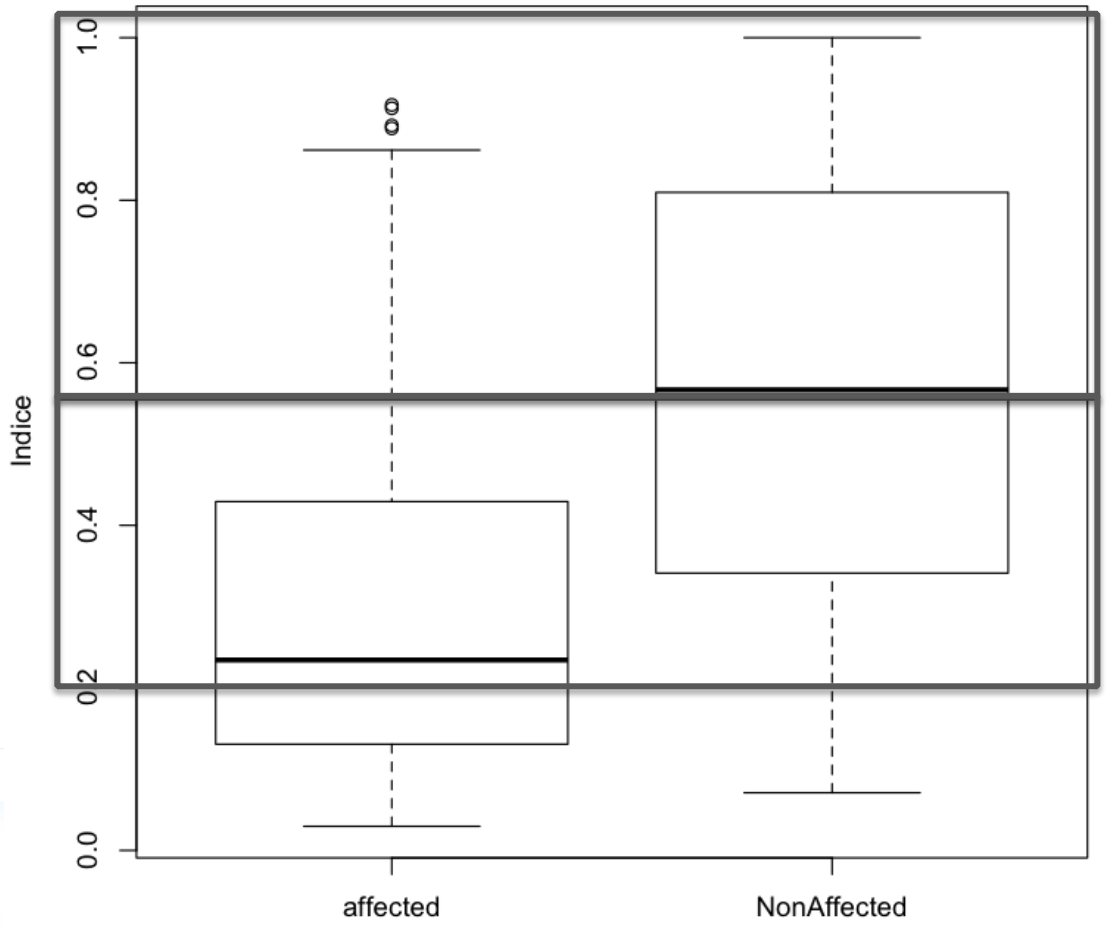
Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

INDEX A

47 % of healthy
10% of affected

INDEX B

43 % of healthy
50% of affected



p-value : 1.754 10-16 (Mann Whitney test)

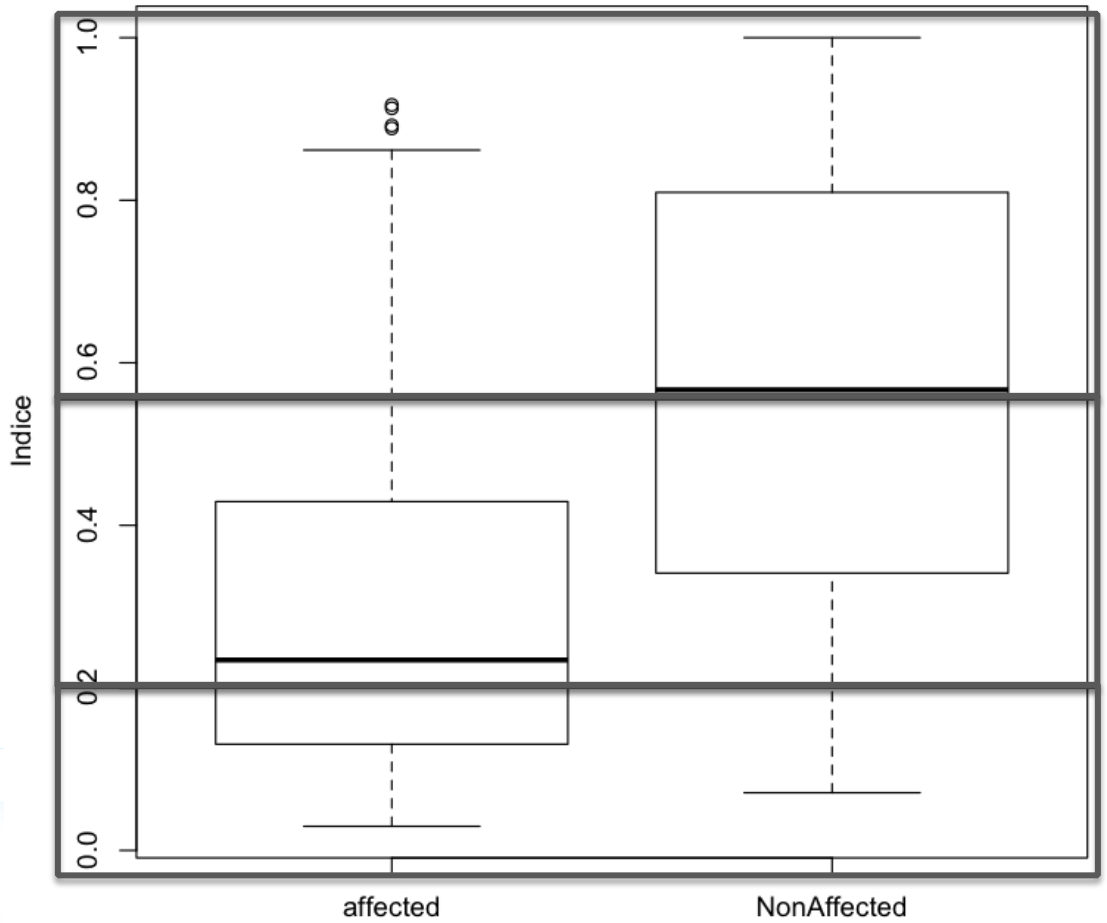
Development of a genetic risk test for breeders
Definition of an Index

Index distribution in case and control populations on 256 affected BMDs and 165 unaffected

INDEX A

INDEX B

INDEX C



47 % of healthy
10% of affected

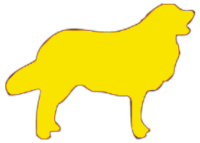
43 % of healthy
50% of affected

10 % of healthy
40% of affected

p-value : 1.754 10-16 (Mann Whitney test)

Development of a genetic risk test for breeders
Definition of an Index

11 11 11 11 11 11 11 11 11 → Index **A**



12 12 12 12 12 12 12 12 12 → Index **B**

22 22 22 22 22 22 22 22 22 → Index **C**

Development of a genetic risk test for breeders

Validation of the A, B, C index:

In collaboration with the European breeder clubs:

validation of the "pre-test" on European BMDs

Belgium, Danemark, Finland, Germany, Ireland, Italia, Poland, Portugal, Spain, Sweden, Switzerland) :

- 80 dogs with Histiocytic Sarcoma
- 64 unaffected dogs (> 10 years)

	A	B	C
Affected	***6.25%***	***33.75%***	***60%***
Healthy	***30.16%***	***47.62%***	***22.22%***

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77%

- **Pre test validated for European BMDs**

Development of a genetic risk test for breeders
Validation of the A, B, C index:

In collaboration with the American :

Berner Garde and Dr Vilma Yuzbasiyan-Gurkan (Michigan State University)

- 54 dogs with Histiocytic Sarcoma
- 37 unaffected dogs (> 10 years)

	A	B	C
Affected	***9.26%***	***50%***	***40.74%***
Healthy	***35.14%***	***48.65%***	***16.22%***

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83%

- Pre test validated for American BMDs : -> test name has been changed to HS test

Development of a genetic risk test for breeders
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83%

- **Pre test validated for American BMDs : -> test name has been changed to HS test**

Development of a genetic risk test for breeders
Validation of the A, B, C index:

In total, **718** dogs used for the development and validation of the pre-test

- ➢ 415 dogs with Histiocytic Sarcoma
- ➢ 303 unaffected dogs (> 10 years) + breeders and Club initiatives

	A	B	C
Affected	***14%***	***43.1%***	***42.9%***
Healthy	***41.5%***	***46.5%***	***11.9%***

➢ **Statistics :** 88%

Index A to detect healthy dogs :

Specificity, ie **probability that an affected dog is not A : 86%**

Index C to detect affected dogs :

Specificity, ie **probability that a healthy dog is not C : 88%**

Summary of SH tests performed by Antagene

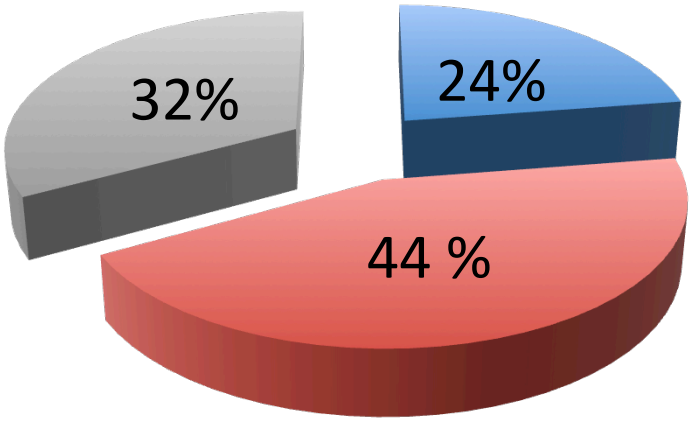
Since 2012 , Antagene has tested **2790 dogs** (unknown phenotypes)

Check the correlation between the index and known clinical status

Summary of SH tests performed by Antagene

Since 2012 , Antagene has tested **2790 dogs** (unknown phenotypes)

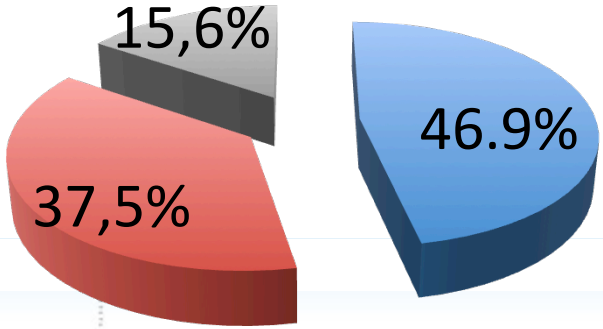
Distribution of index in this population:



A 24 %
B 44 %
C 32%

Work with C dogs !
Mate with A dogs !

Distribution among 64 dogs >10 years old



A 47 % - 30 dogs
B 37,5% - 24 dogs
C 15,5% - 10 dogs

➢ **Good correlation** of the index and known clinical status

Summary of SH tests performed by Antagene

Since 2012 , Antagene has tested nearly 3000 dogs (exact 2790)

In France : 1257 BMDs : 25% A ; 44% B ; 31% C
In Europe (not France) : 1422 BMDs : 24% A ; 45% B ; 31% C

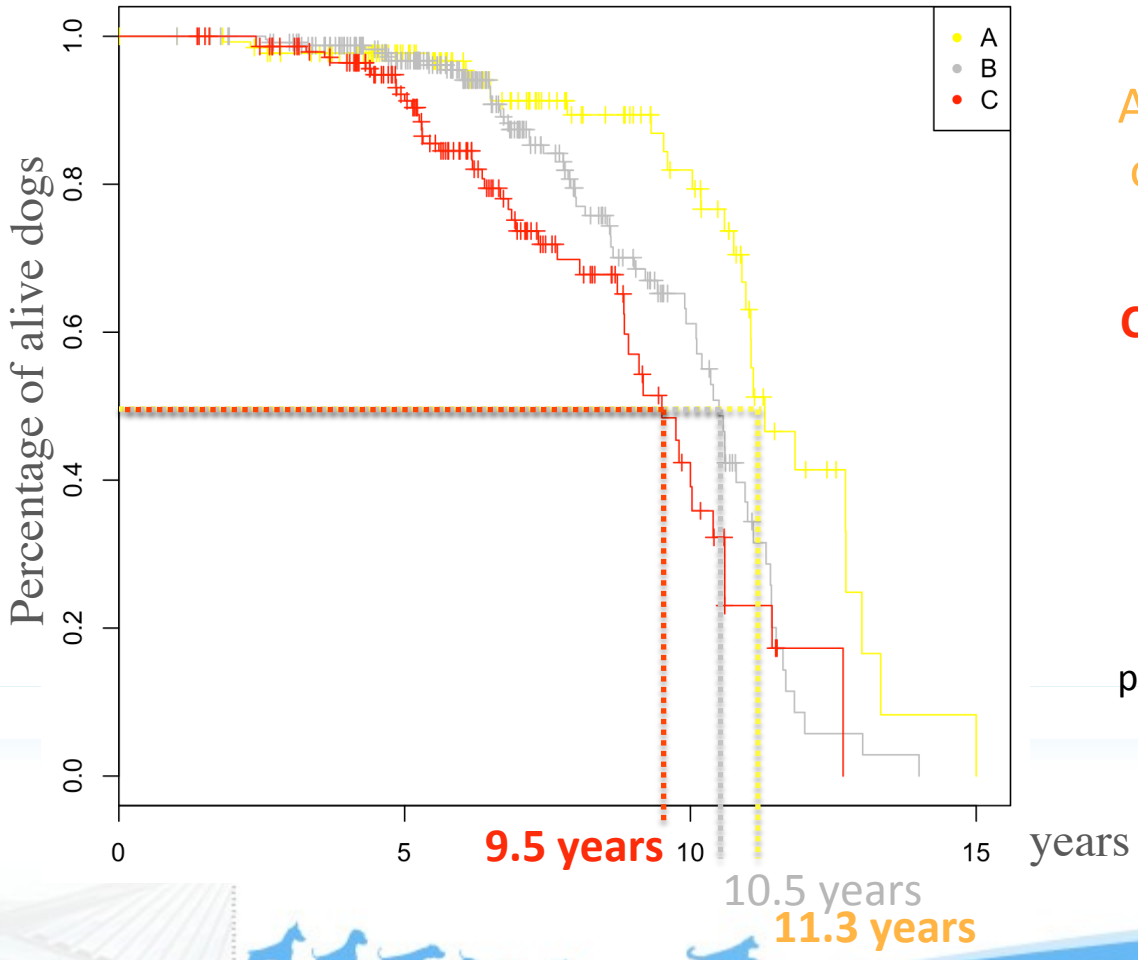
Switzerland : 336 BMDs,
Sweeden : 243 BMDs
Norway : BMDs

Identical A, B, C percentages

IN US : SH genetic risk test is available through Antgene/Optigen recently

Summary of tests performed by Antagene
2000 SH genetic risk tests at end 2017

2012-2017 : Follow up of >5 years old dogs (Vet PhD Eléonore Thiery)



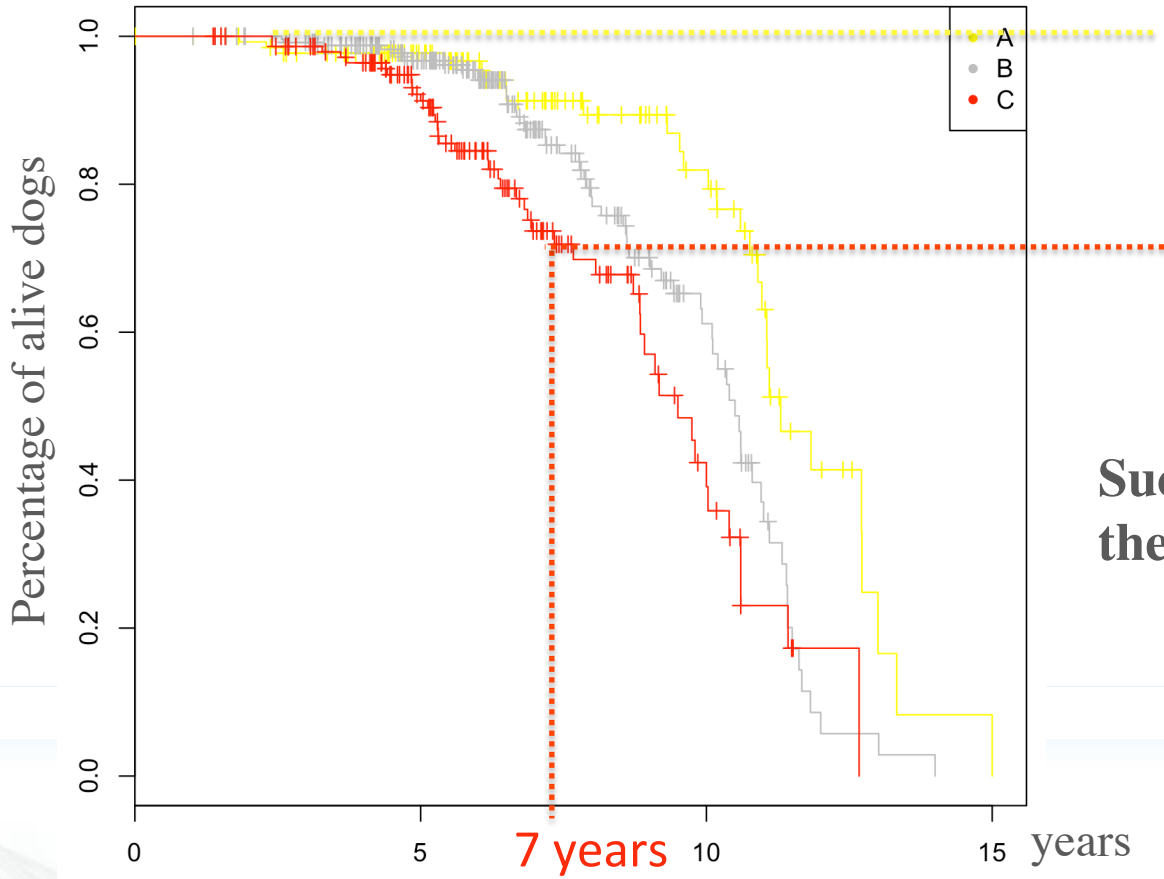
A dogs die at a mean of 11.3 years

C dogs die at a mean of 9.5 years

pval = 3.791686e-06

Summary of tests performed by Antagene
2000 SH genetic risk tests at end 2017

2012-2017 : Follow up of >5 years old dogs (Vet PhD Eléonore Thiery)



A: 15,1% of deaths

B: 35,8% of deaths

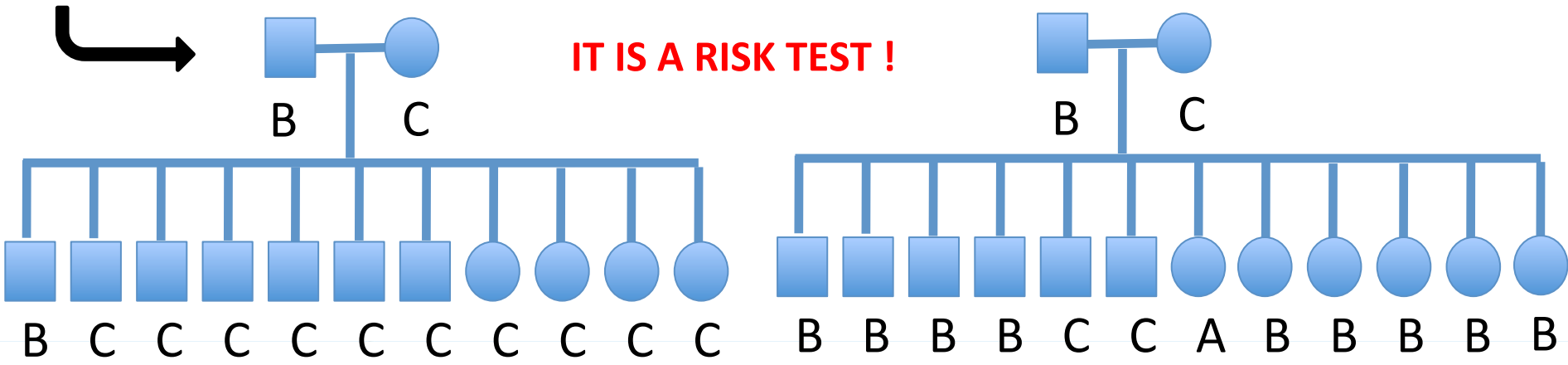
C: 49,1% of deaths

Such a follow up shows that the SH test is robust

Summary of the SH genetic risk test use in complete litters

In general, index of puppies corresponds to average of parents' index

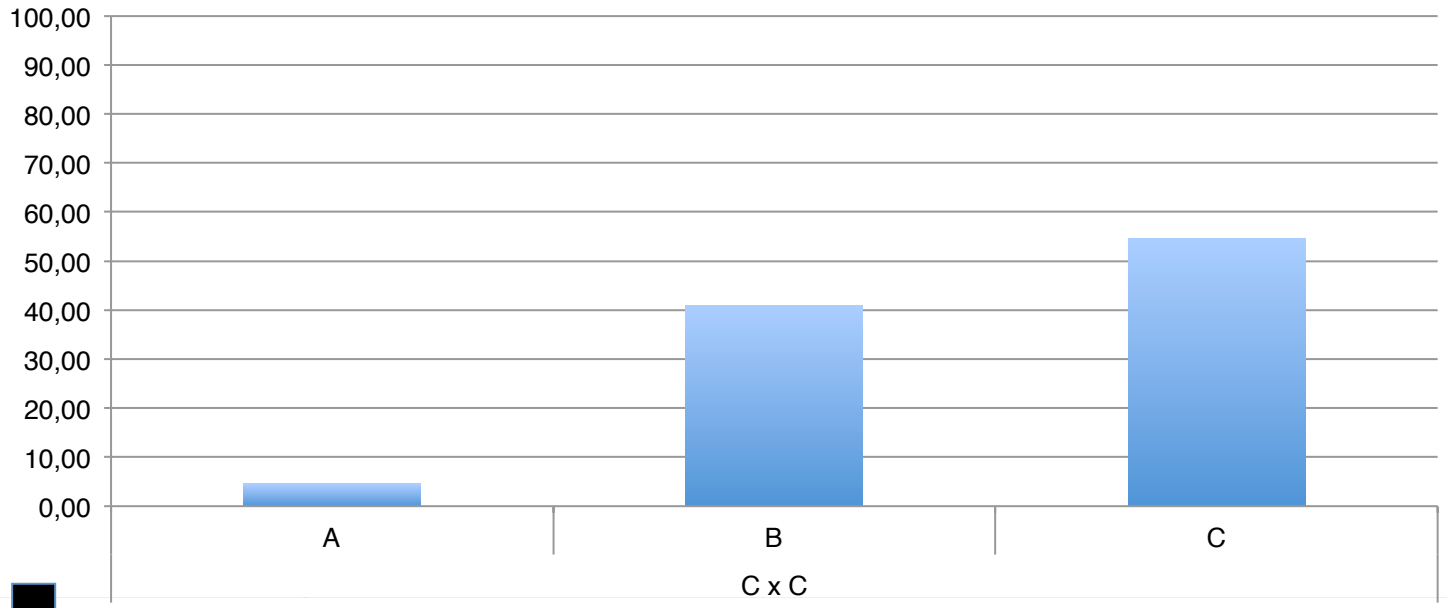
- Exceptionally A x A matings can produce C puppies
- Exceptionally C x C matings can produce A puppies
- 2 matings with identical index can produce different index distributions.



SH genetic risk test : Study of Index in complete litters

In collaboration with French club (AFBS), Antagene, we tested : 112 puppies from 18 litters:

➢ Mating : C x C

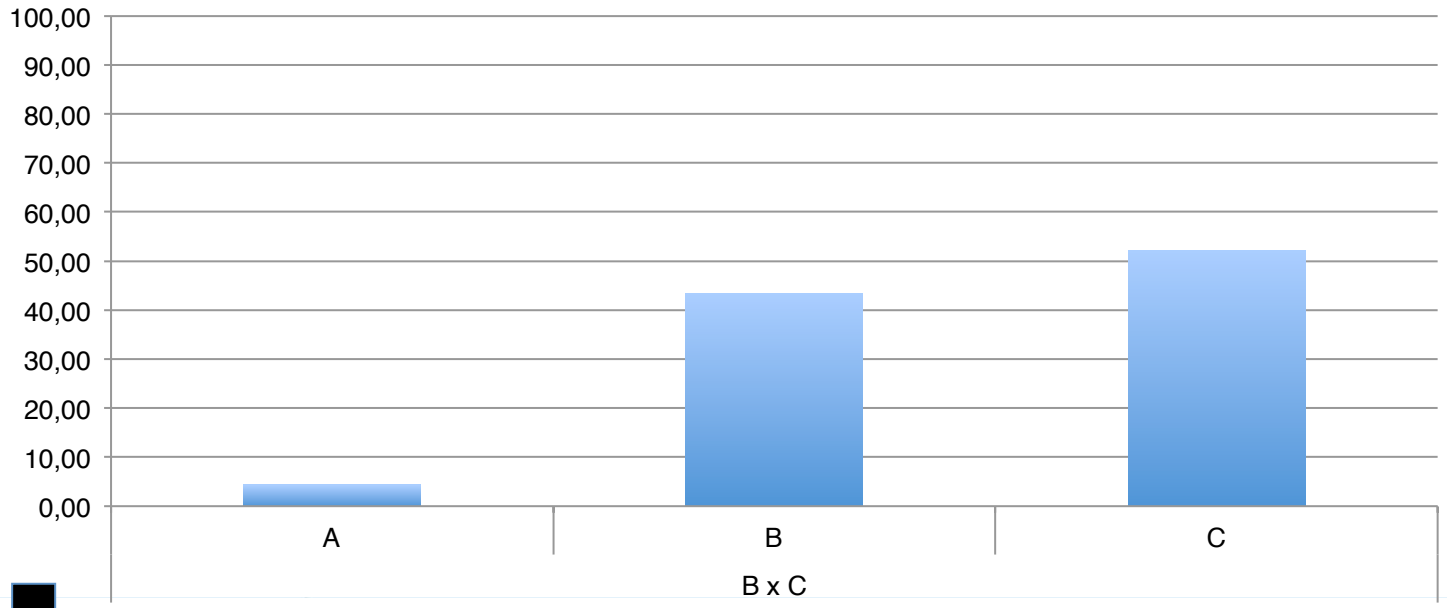


4 litters – 22 dogs
4.5 % of A
41 % of B
54.5% of C

SH genetic risk test : Study of Index in complete litters

In collaboration with French club (AFBS), Antagene, we tested : 112 puppies from 18 litters:

➢ Mating : B x C

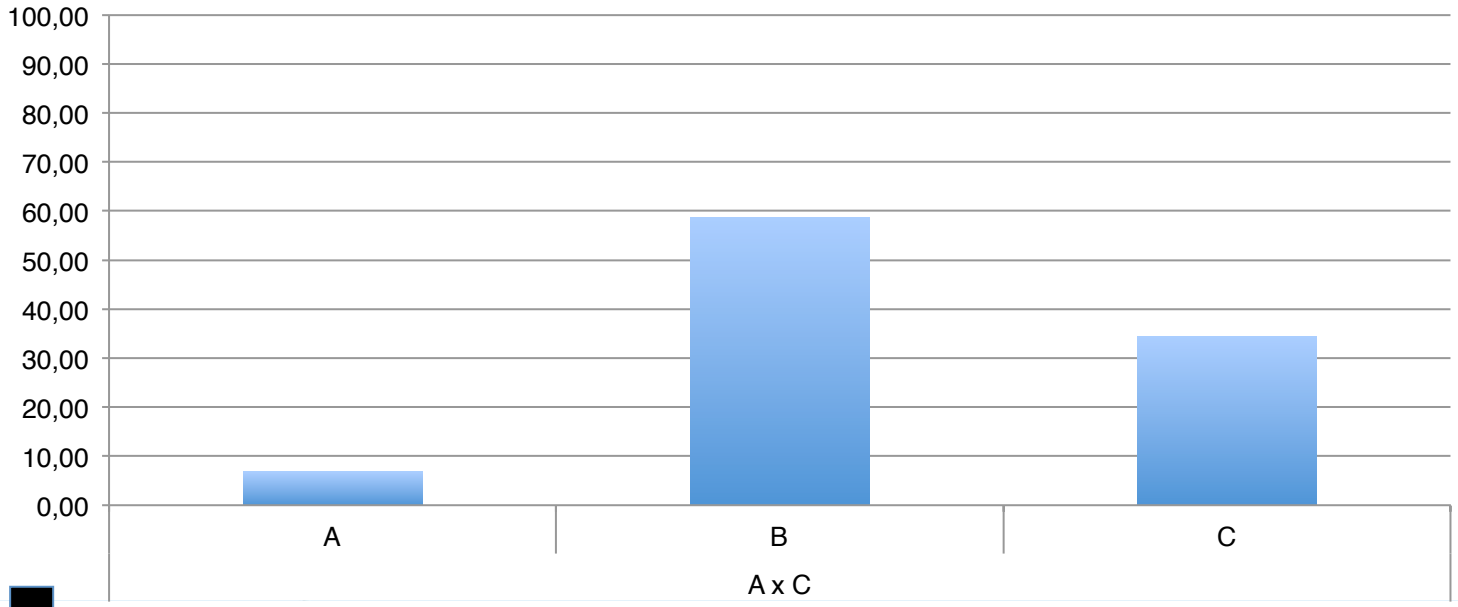


2 litters – 23 dogs
4.4 % of A
43.5 % of B
52,1% of C

SH genetic risk test : Study of Index in complete litters

In collaboration with French club (AFBS), Antagene, we tested : 112 puppies from 18 litters:

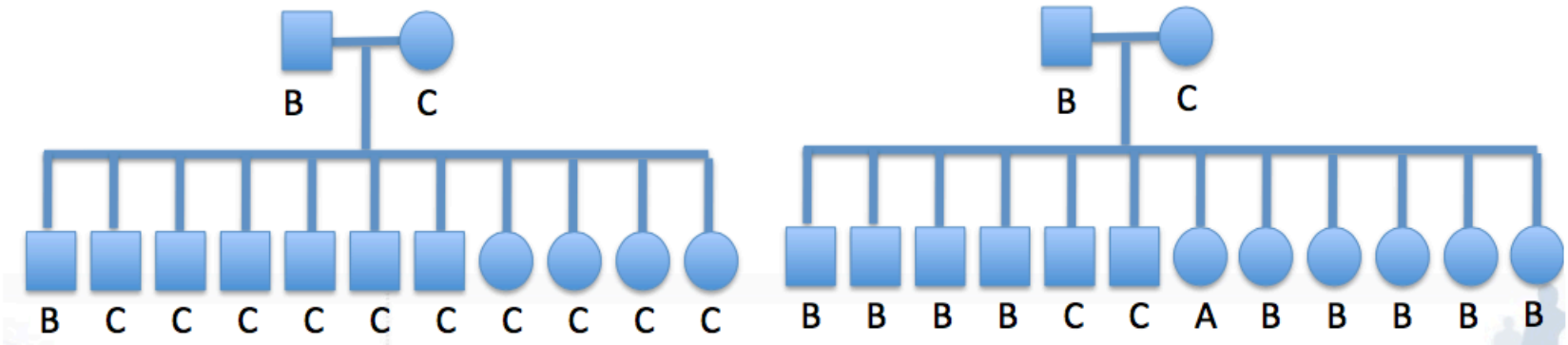
➢ Mating : A x C



6 litters – 29 dogs
7% of A
58.5 % of B
34.5% of C

Development of HSIMS : Collaboration between CNRS and Antagene

➢ The compatibility between indexes varies depending on the genetics of each parent



➢ Idea to develop a statistical test based on all possible allele combination for a given mating and prediction of the SH risk for the puppies

AUSTRIA, FRANCE, SLOVAKIA, SLOVENIA, SWEDEN, SWITZERLAND, USA

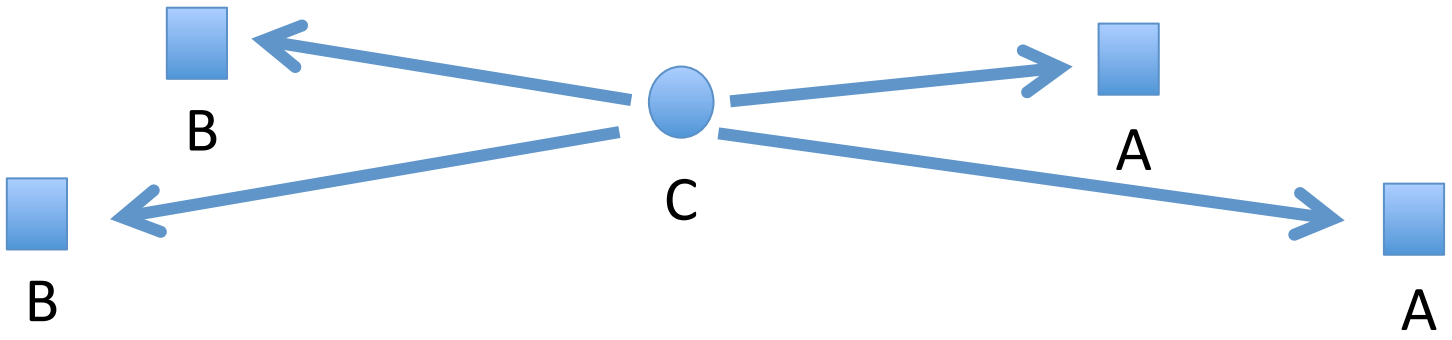
participated to develop this companion test, soon available at Antagene

Development of HSIMS : Collaboration between CNRS and Antagene

C

HSIMS : Histiocytic Sarcoma Index Mate Selection

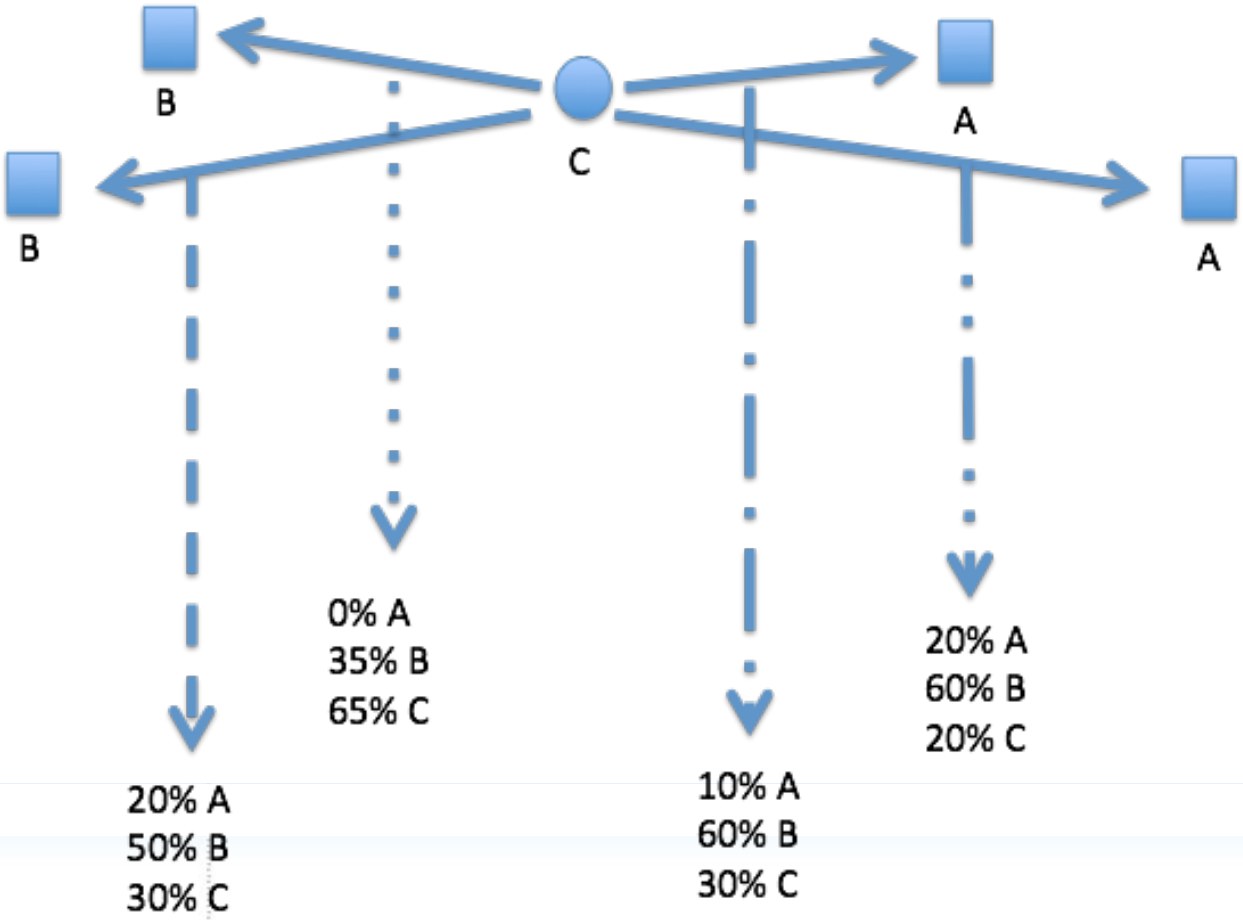
Development of HSIMS : Collaboration between CNRS and Antagene



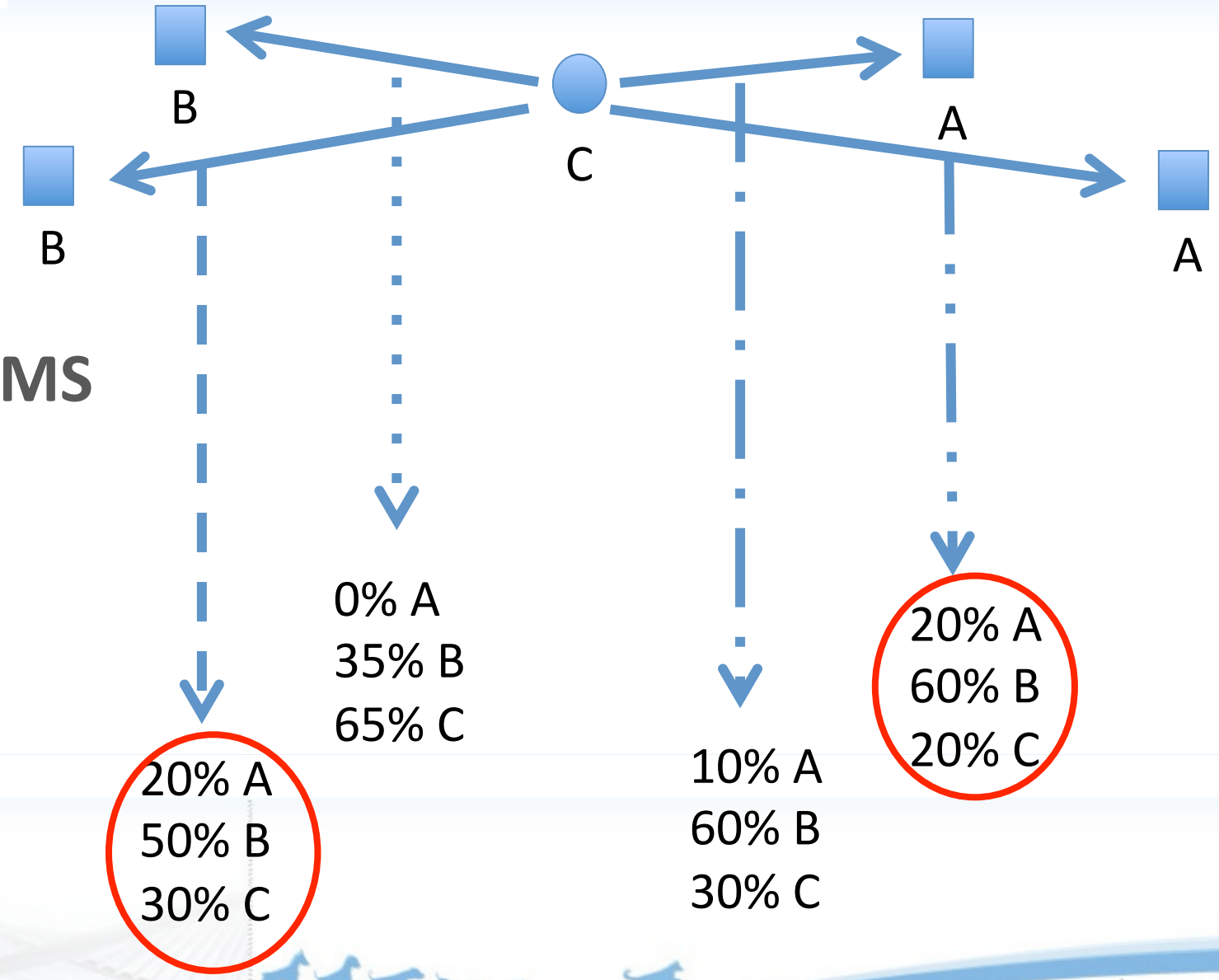
HSIMS : Histiocytic Sarcoma Index Mate Selection

Helps to select the best mating (for HS susceptibility)

Development of HSIMS : Collaboration between CNRS and Antagene



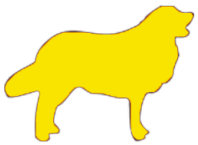
Development of HSIMS : Collaboration between CNRS and Antagene

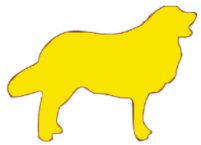


Development of HSIMS : Collaboration between CNRS and Antagene

Histiocytic Sarcoma Index Mate Selection

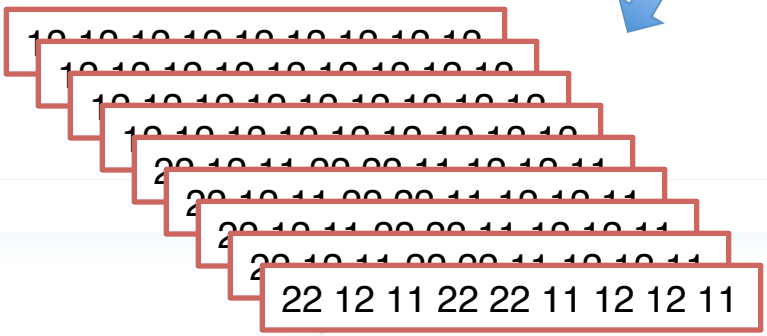
With the 9 markers of the actual SH Genetic Risk Test :





12 22 11 12 12 22 11 12 12

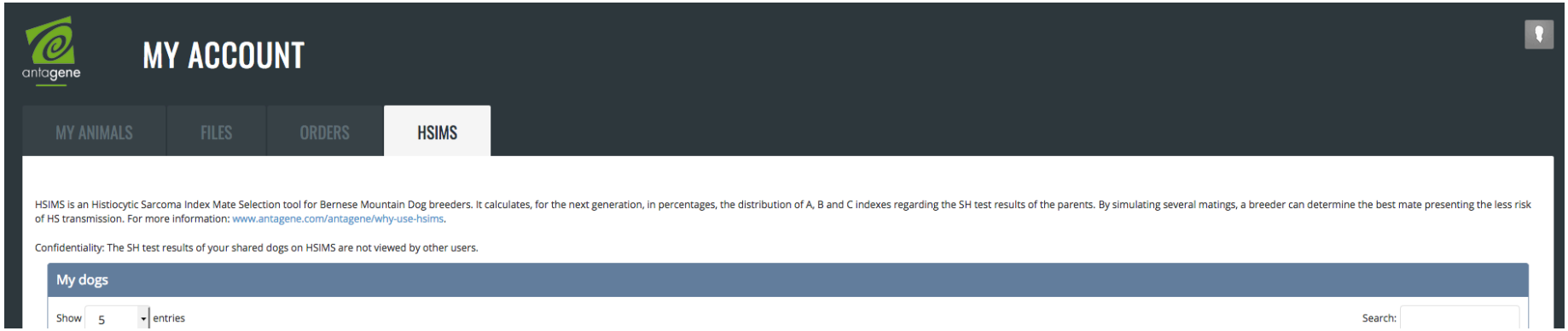
22 12 12 11 22 12 11 12 12



Some **20 000** possibilitiess
For 1 mating :
Need to automate the estimations !

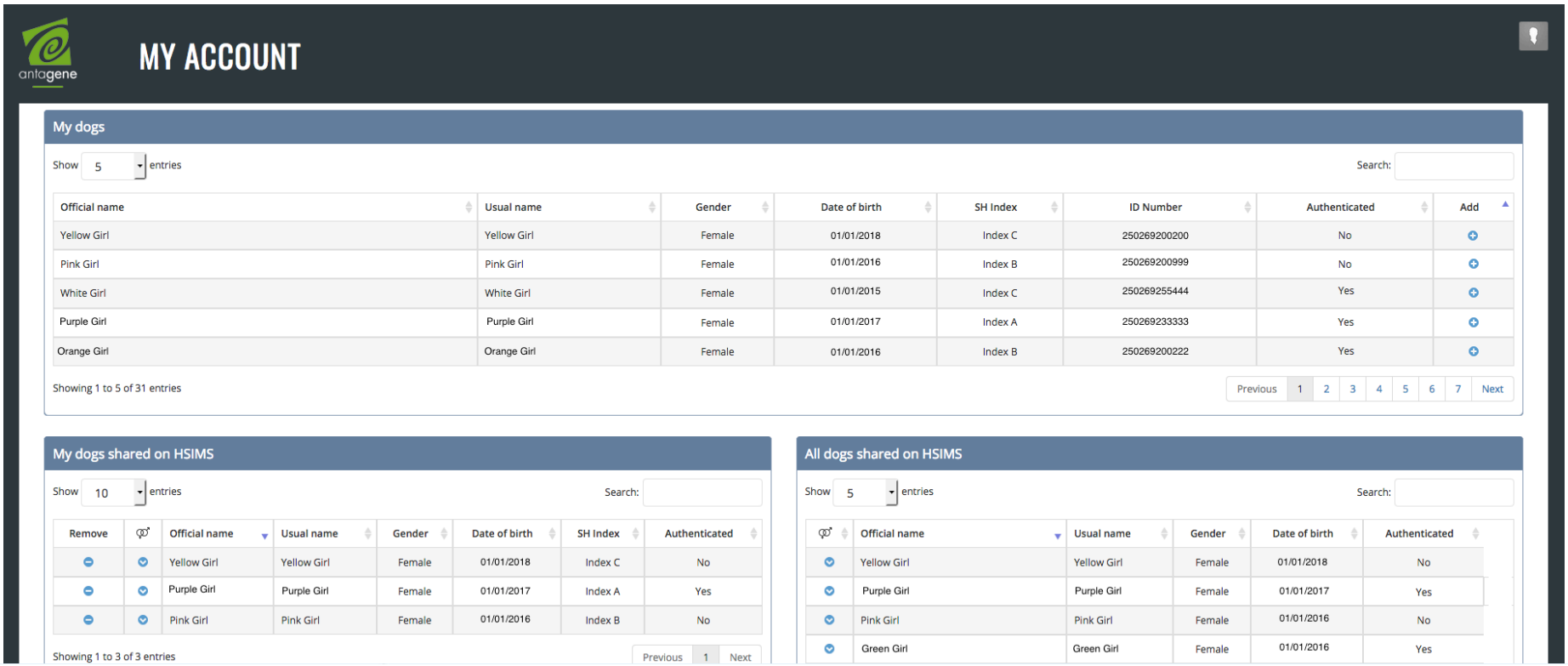
Development of HSIMS : Collaboration between CNRS and Antagene

HSIMS tool will be available into your ANTAGENE account
Several Tables :



Development of HSIMS : Collaboration between CNRS and Antagene

Table containing all your dogs tested, dogs added in HSIMS, all dogs in HSIMS



MY ACCOUNT

My dogs

Show 5 entries — Search:

Official name	Usual name	Gender	Date of birth	SH Index	ID Number	Authenticated	Add
Yellow Girl	Yellow Girl	Female	01/01/2018	Index C	250269200200	No	
Pink Girl	Pink Girl	Female	01/01/2016	Index B	250269200999	No	
White Girl	White Girl	Female	01/01/2015	Index C	250269255444	Yes	
Purple Girl	Purple Girl	Female	01/01/2017	Index A	250269233333	Yes	
Orange Girl	Orange Girl	Female	01/01/2016	Index B	250269200222	Yes	

Showing 1 to 5 of 31 entries — Previous 1 2 3 4 5 6 7 Next

My dogs shared on HSIMS

Show 10 entries — Search:

Remove		Official name	Usual name	Gender	Date of birth	SH Index	Authenticated
		Yellow Girl	Yellow Girl	Female	01/01/2018	Index C	No
		Purple Girl	Purple Girl	Female	01/01/2017	Index A	Yes
		Pink Girl	Pink Girl	Female	01/01/2016	Index B	No

Showing 1 to 3 of 3 entries — Previous 1 Next

All dogs shared on HSIMS

Show 5 entries — Search:

	Official name	Usual name	Gender	Date of birth	Authenticated
	Yellow Girl	Yellow Girl	Female	01/01/2018	No
	Purple Girl	Purple Girl	Female	01/01/2017	Yes
	Pink Girl	Pink Girl	Female	01/01/2016	No
	Green Girl	Green Girl	Female	01/01/2016	Yes

Development of HSIMS : Collaboration between CNRS and Antagene

Mating before selection of the parents

My dogs shared on HSIMS

Show 10 entries Search:

Remove		Official name	Usual name	Gender	Date of birth	SH Index	Authenticated
⊖	⊙	Yellow Girl	Yellow Girl	Female	01/01/2018	Index C	No
⊖	⊙	Purple Girl	Purple Girl	Female	01/01/2017	Index A	Yes
⊖	⊙	Pink Girl	Pink Girl	Female	01/01/2016	Index B	No

Showing 1 to 3 of 3 entries Previous 1 Next

All dogs shared on HSIMS

Show 5 entries Search:

	Official name	Usual name	Gender	Date of birth	Authenticated
⊙	Yellow Girl	Yellow Girl	Female	01/01/2018	No
⊙	Purple Girl	Purple Girl	Female	01/01/2017	Yes
⊙	Pink Girl	Pink Girl	Female	01/01/2016	No
⊙	Green Girl	Green Girl	Female	01/01/2016	Yes
⊙	Blue Boy	Blue Boy	Male	01/01/2016	Yes

Showing 1 to 5 of 10 entries Previous 1 2 Next

Selected mating

Select a dog from your kennel and another dog of the opposite gender from all shared dogs on HSIMS to test their compatibility

Development of HSIMS : Collaboration between CNRS and Antagene

Mating after selection of the parents (you have to be the owner of one dog at least

My dogs shared on HSIMS

Show 10 entries Search:

Remove		Official name	Usual name	Gender	Date of birth	SH Index	Authenticated
⊖	⊙	Yellow Girl	Yellow Girl	Female	01/01/2018	Index C	No
⊖	⊙	Purple Girl	Purple Girl	Female	01/01/2017	Index A	Yes
⊖	⊙	Pink Girl	Pink Girl	Female	01/01/2016	Index B	No

Showing 1 to 3 of 3 entries Previous 1 Next

All dogs shared on HSIMS

Show 5 entries Search:

	Official name	Usual name	Gender	Date of birth	Authenticated
⊙	Yellow Girl	Yellow Girl	Female	01/01/2018	No
⊙	Purple Girl	Purple Girl	Female	01/01/2017	Yes
⊙	Pink Girl	Pink Girl	Female	01/01/2016	No
⊙	Green Girl	Green Girl	Female	01/01/2016	Yes
⊙	Blue Boy	Blue Boy	Male	01/01/2016	Yes

Showing 1 to 5 of 10 entries Previous 1 2 Next

Selected mating

Purple Girl	**Blue Boy**
Date of birth : 01/01/2017	Date of birth : 01/01/2016
Female	Male
Authenticated	Authenticated
Owner : **Ms CNRS**	Owner : **Mr ANTAGENE**

Start the simulation

Development of HSIMS : Collaboration between CNRS and Antagene

Simulations and calculation of the percentages of A, B, C puppies

Selected mating

Purple Girl	Blue Boy
Date of birth : 01/01/2017	Date of birth : 01/01/2016
Female	Male
Authenticated	Authenticated
Owner : Ms CNRS	Owner : Mr ANTAGENE

Start the simulation

Index A	0%
Index B	6%
Index C	94%

Download the simulation

Selected mating

Purple Girl	Grey Boy
Date of birth : 01/01/2017	Date of birth : 01/01/2016
Female	Male
Authenticated	Authenticated
Owner : Ms CNRS	Owner : Mr ANTAGENE

Start the simulation

Index A	30%
Index B	47%
Index C	23%

Download the simulation

Development of HSIMS : Collaboration between CNRS and Antagene

Results
in PDF format

antagene

HSIMS Simulation

Female	Male
Name : Purple Girl	Name : Grey Boy
ID Number : 250269000099999	ID Number : 250269000000000
Date of birth : 01/01/2017	Date of birth : 01/01/2016
Sample number : 777777	Sample number : 999999

Statistical distribution of the SH indexes in the progeny :

Index A	30%
Index B	47%
Index C	23%

Explanation

HSIMS (Histiocytic Sarcoma Index Mate Selection) is a tool for mating selection according to the SH index of the parents. This selection tool allows to statistically calculate on the progenies the probabilities to obtain A, B or C index for a given mating. It is based exhaustively on all possible SH genotypes in the progeny. Thus, the result of the HSIMS tool is strictly specific to each considered couple. The performed simulation is exhaustive and reliable but may not accurately reflect reality on a litter scale.

Ongoing research on canine histiocytic sarcomas

Sample collection → Clinical, Histological, Epidemiological → Molecular characterization - predisposition - somatic → Gene Identification, Gene function

Cani-DNA

Homologies with Human sarcomas

Transfer to Human Translational research

→Identifiction of predisposition loci : 3 major chromosomes

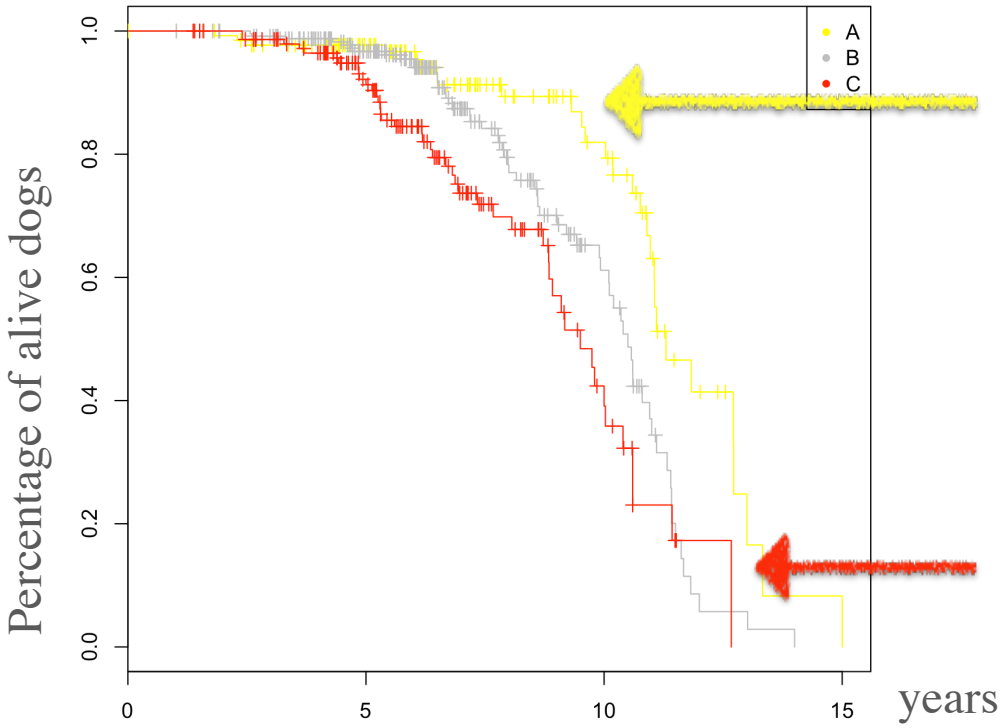
→ SH genetic risk test + HSIMS

→ improvement of the present SH genetic risk test

Abadie, Hédan et al., 2009;
Hédan et al., 2011;
Shearin, Hedan et al., 2012
Rault, Hedan et al. In Prep

- Identification of somatic mutations (in the tumour) in an oncogene (in humans too !)
- Functional analyses ongoing → Research on a diagnostic « blood test »
- Development of 8 canine sarcoma cell lines → to perform drug tests

Ongoing research to improve the SH genetic risk test
The main question :



➢ Why ~14 % of affected are A?

➢ Why ~12% of unaffected are C?

→ SH Test improvement by selecting and including more markers and

→ By analyzing discordant dogs

Conclusion

Test HS : first genetic risk test in veterinary cancer

- In full collaboration with breeders and Clubs, step by step
- Development, Pilot study, Validation, Follow up (2012-2018)
- 2018 : Nearly 3000 BMDs tests by ANTAGENE

-> research is still needed to improve this test!

- Improvement of the SH genetic risk test
- Mutations in tumors : useful markers for an earlier diagnosis, for treatment

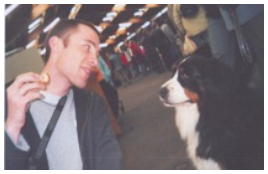
inform Antagene of dogs' outcome: online clinical follow-up questionnaire : http://www.antagene.com/en/contact/histiocytic-sarcoma-questionnaire\

Contact us in case of suspicion : benoit.hedan@univ-rennes1.fr

Always looking for samples (suspicion/cases…) to pursue research!

Acknowledgments

Canine Genetics Team
Catherine Andre et al.
CNRS Rennes France



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Vilma Yuzbasiyan-Gurkan

Owners, breeders, Vets
Histopathology labs :
LAPVSO and IDEXX

Fundings : **BMD clubs : AFBS, SIBB, DCBS...**

Clubs, Berner Garde,...
Pat Long encouragments