HISTIOCYTIC SARCOMA IN DOGS

Genetic progress in the fight against histiocytic sarcoma
last update of the genetic risk test : HS Pre-test

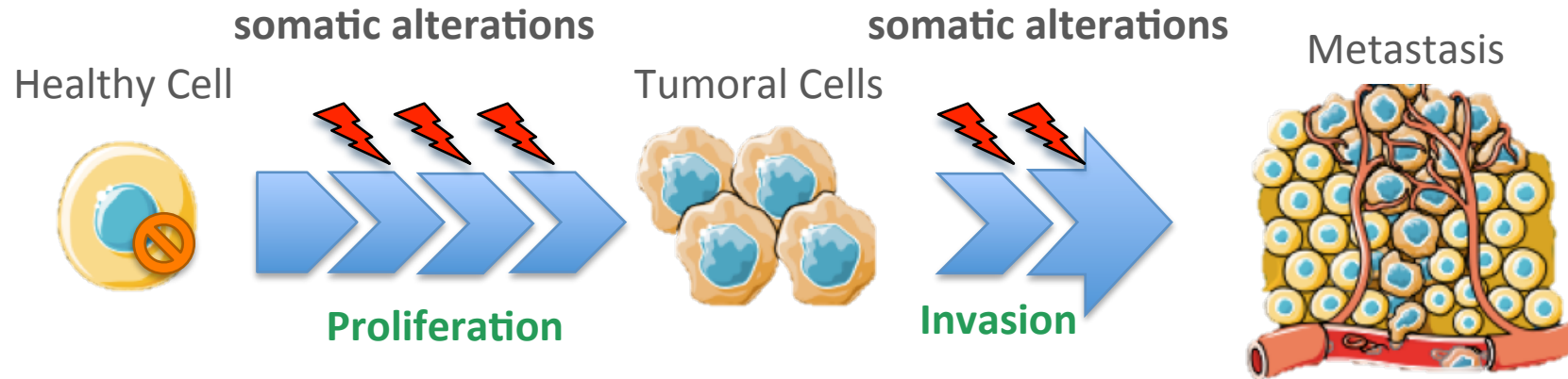## Benoit Hedan
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Collaboration with :
**Anne Thomas and Caroline Dufaure de Citres**
ANTAGENE

Long term goal :
Identify genetic mechanisms involved in HS

somatic alterations   somatic alterations

Healthy Cell   Tumoral Cells   Metastasis

Proliferation   **Invasion**

➢ Identify predisposing genes and risk alleles : Blood collection

➢ Identify recurrent somatic alterations associated with tumor progression :
Tumor tissue

Tissu tumoral

Tissu sain
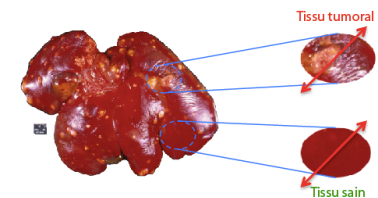
Double benefit for Human and veterinarian medicine

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Long term collaboration :
Identify genetic mechanisms involved in HS

somatic alterations

somatic alterations

Metastasis

Healthy Cell

Tumoral Cells

Proliferation

Invasion
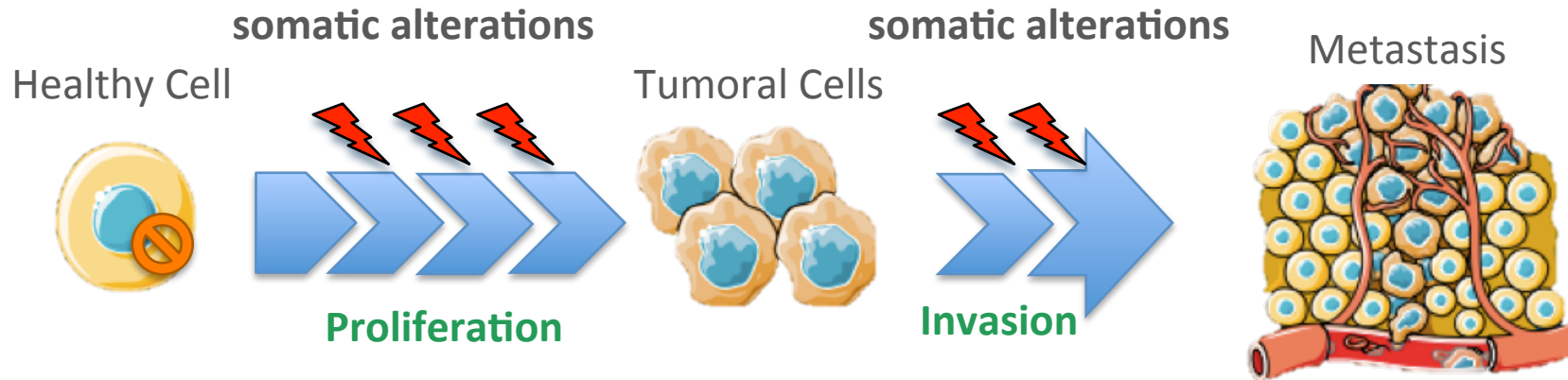
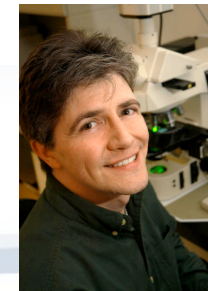➤ Identify predisposing genes and risk alleles :

  E. Ostrander's lab,
  The Cancer genetics branch NIH (Bethesda, USA)

➤ Identify recurrent somatic alterations associated with tumor progression :

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

Looking for predisposing genes:
Research project

➢ Since 2002 - Cani-DNA biobank (dog.genetics.genouest.org)

- > 3000 blood samples and > 300 tissue samples

- > 350 HS cases with histopathological report

➢ Characterization of the Disease:
   Abadie, Hedan et al. 2009
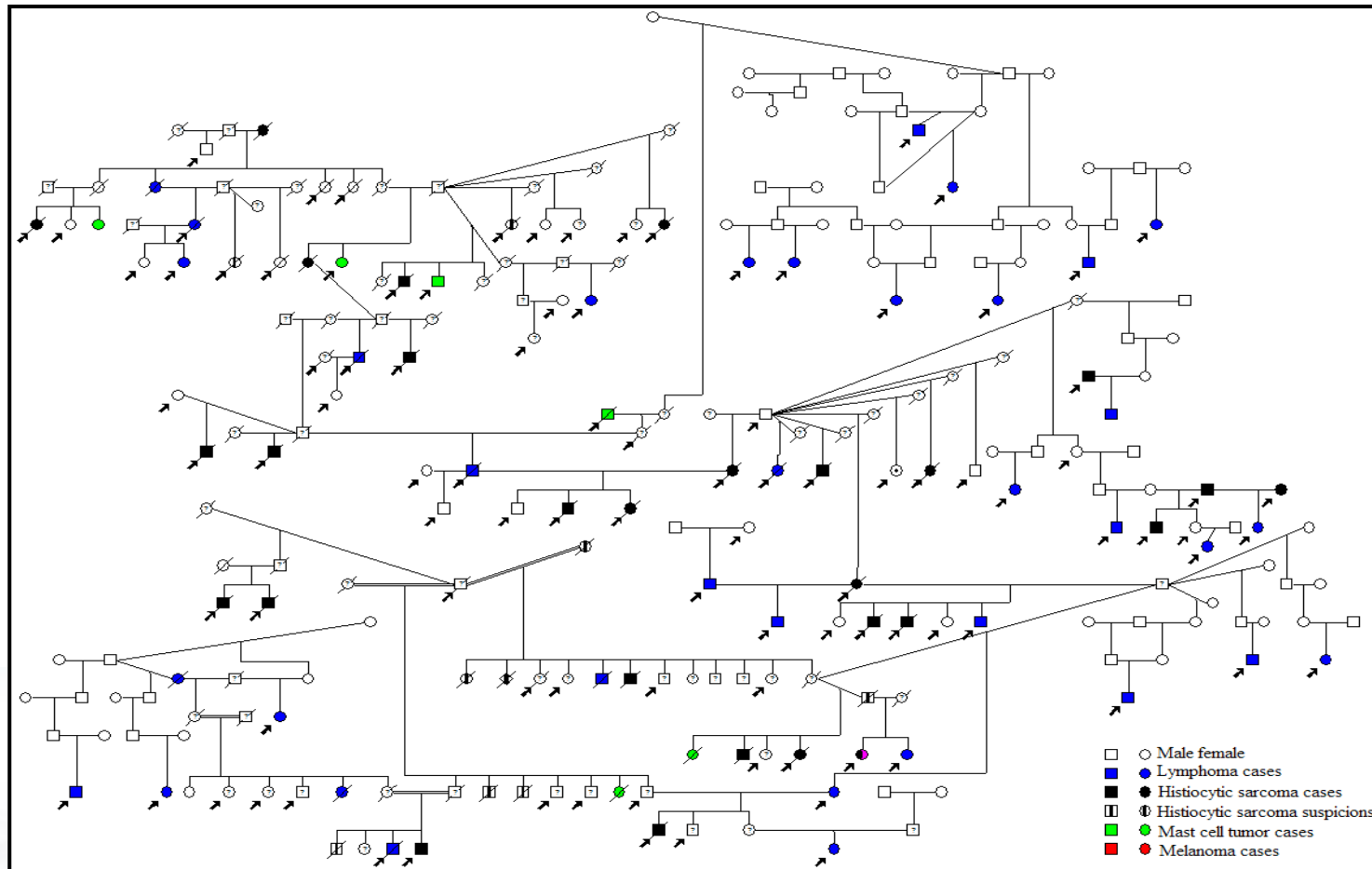

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Legend:
- ☐ ○ Male female
- ■ ● Lymphoma cases
- ■ ● Histiocytic sarcoma cases
- ◧ ◑ Histiocytic sarcoma suspicions
- ■ ● Mast cell tumor cases
- ■ ● Melanoma cases

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➢ Characterization of the Disease:
  Abadie, Hedan et al. 2009

➢ In coll. with E. Ostrander's lab (NIH, Bethesda)

  GWAS (Affymetrix SNP array 22K): 232 unaffected and 244 affected BMDs (American/European)

  -> Identification of main loci : CFA11, CFA14 *(Shearin, Hedan et al. 2012)*

➢ New GWAS (Illumina SNP array 170K) 2011-2012:   164 unaffected and 169 affected BMDs
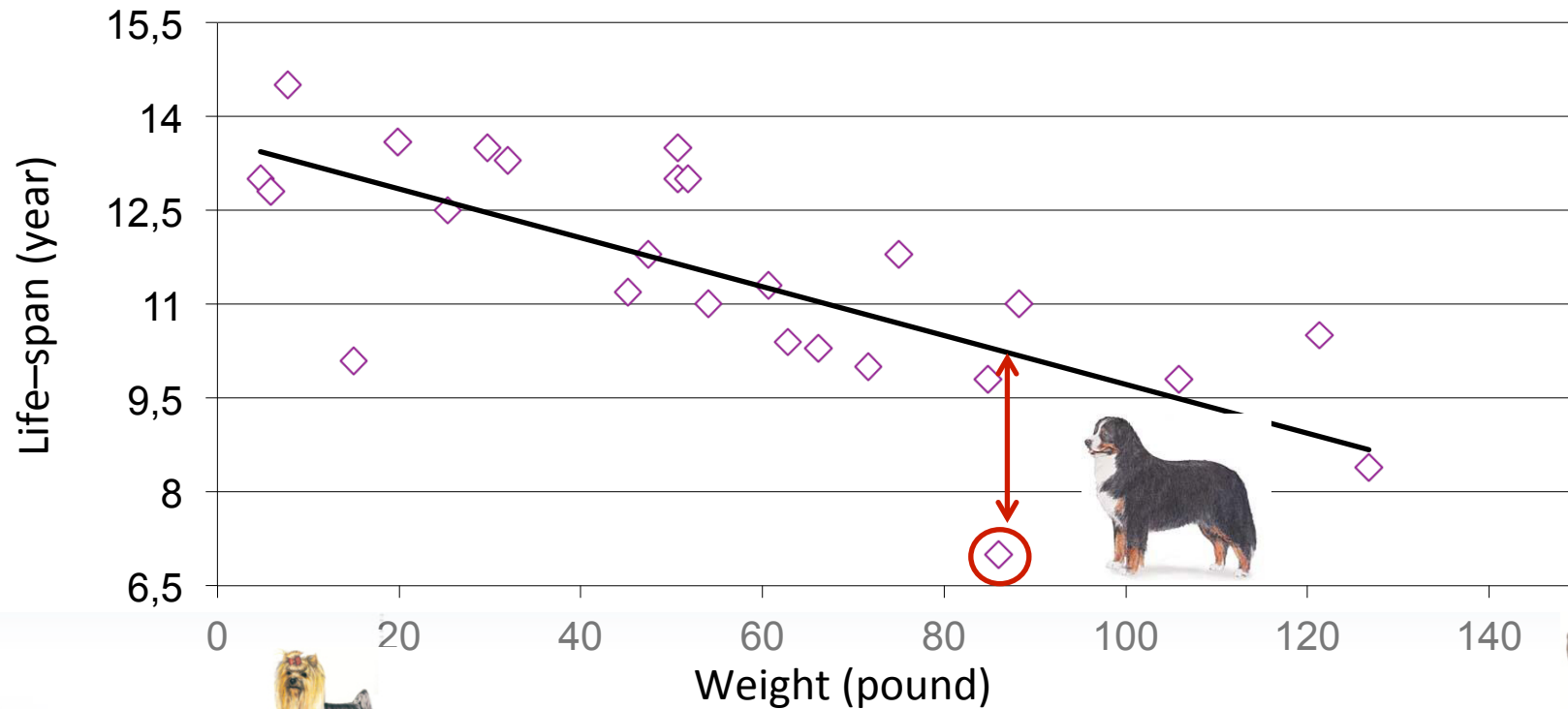
Looking for predisposing genes:
Research project

➤ Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

- Capture and sequencing of 3 loci + whole genome sequencing of affected and healthy dogs

Summary of HS research

➤ Cancers impact BMD life span :

Looking for predisposing genes:
Research project

➢ Work in progress to identify variants associated with HS predisposition (col. With E. Ostrander)

- Capture and sequencing of 3 loci + whole genome sequencing of affected and healthy dogs

Summary of HS research

➢ Cancers impact BMD life span

➢ Cancer : polyfactorial disease -> involving genetic and environmental factors

➢ Many regions are involved in HS predisposition of which CFA11

> Needs of research to better understand development of this cancer
to prevent and treat it

BUT data produced by research could be useful for breeders to help selection!

How these data could be useful for breeders?

In 2012 , in collaboration with Antagene , we decided to develop a genetic risk test

➢ Based on our first GWAS data, selection of 140 SNPs associated with HS

➢ Genotyping of 1081 French BMDs (256 affected dogs + 165 unaffected dogs)

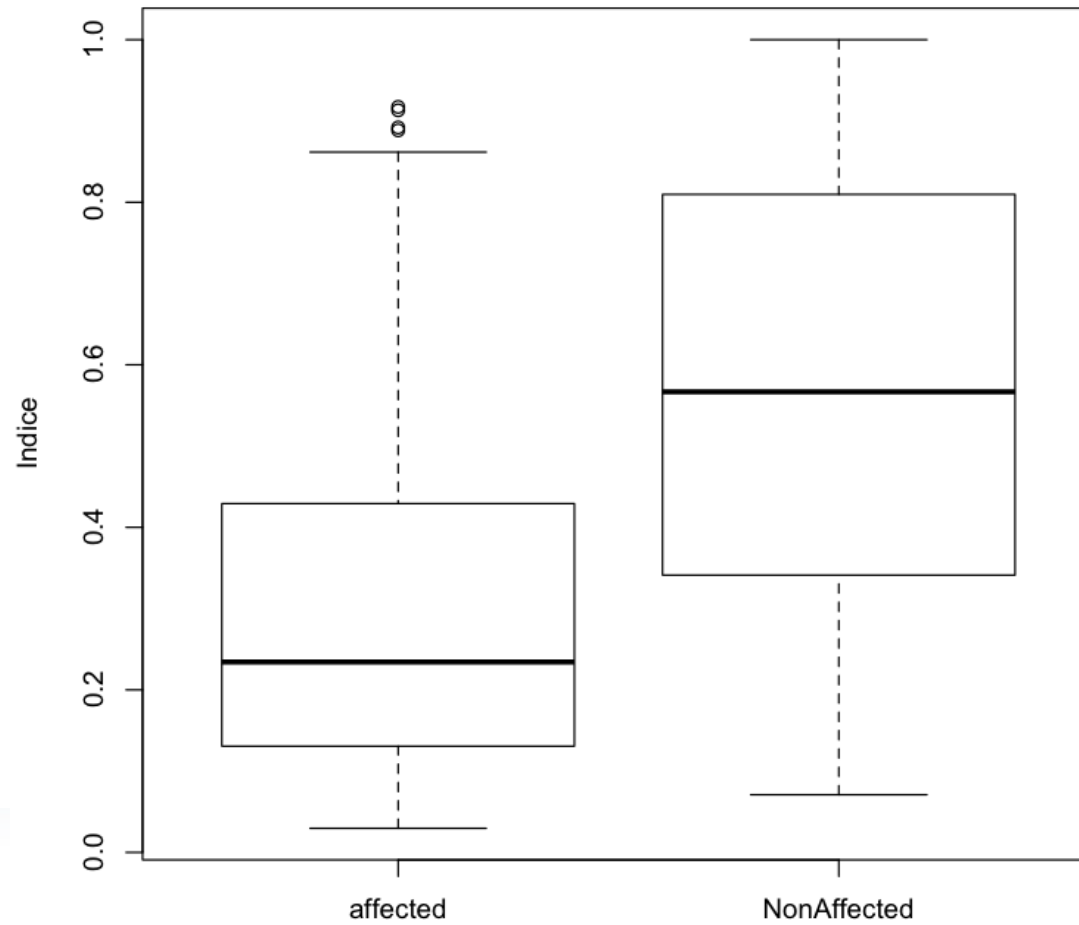   -> selection of the 9 SNPs more significantly associated with risk to develop HS

➢ Estimation of risk to be unaffected (GLM model)   :

$$Risk = \frac{\exp(\overset{n}{\underset{1}{\Sigma}} (\alpha_i \, SNP_i))}{1+\exp(\Sigma (\alpha_i \, SNP_i))}$$

Index

Index development

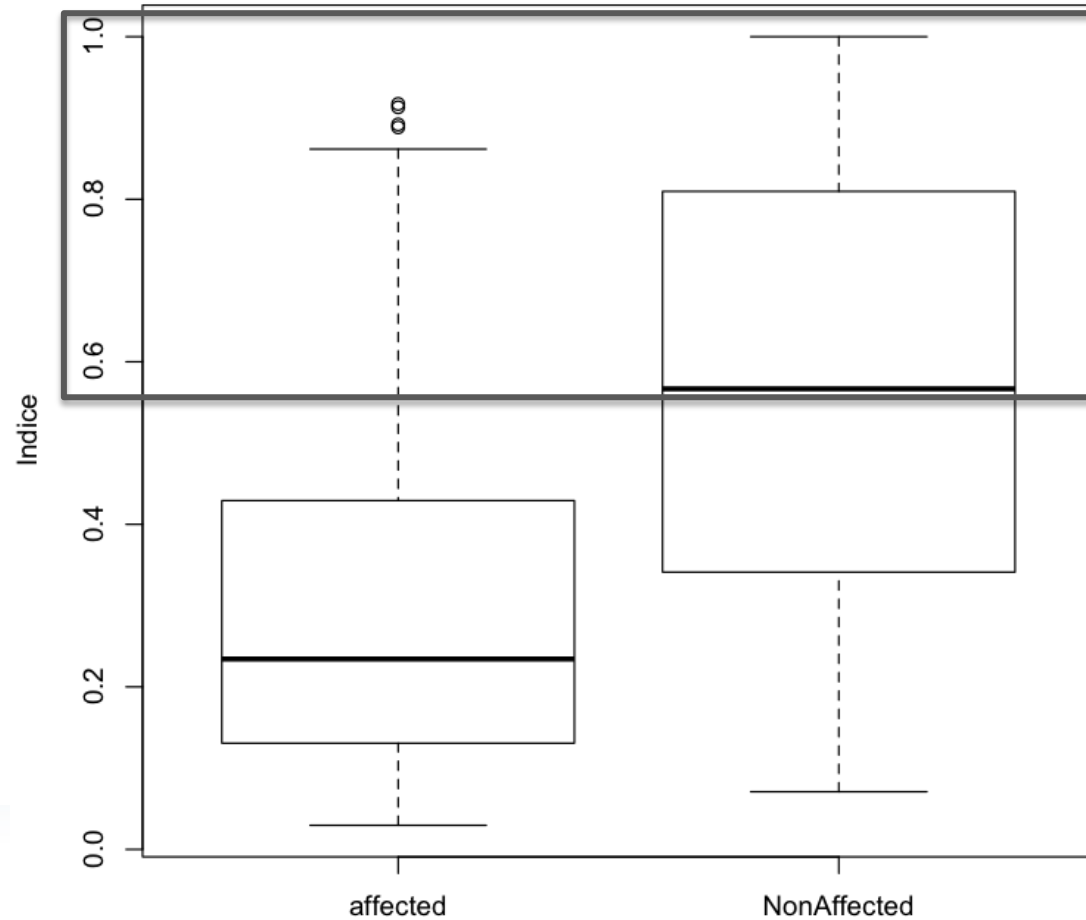Index distribution in case and control populations on 256 affected BMDs and 165 unaffected BMDs

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

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Index development

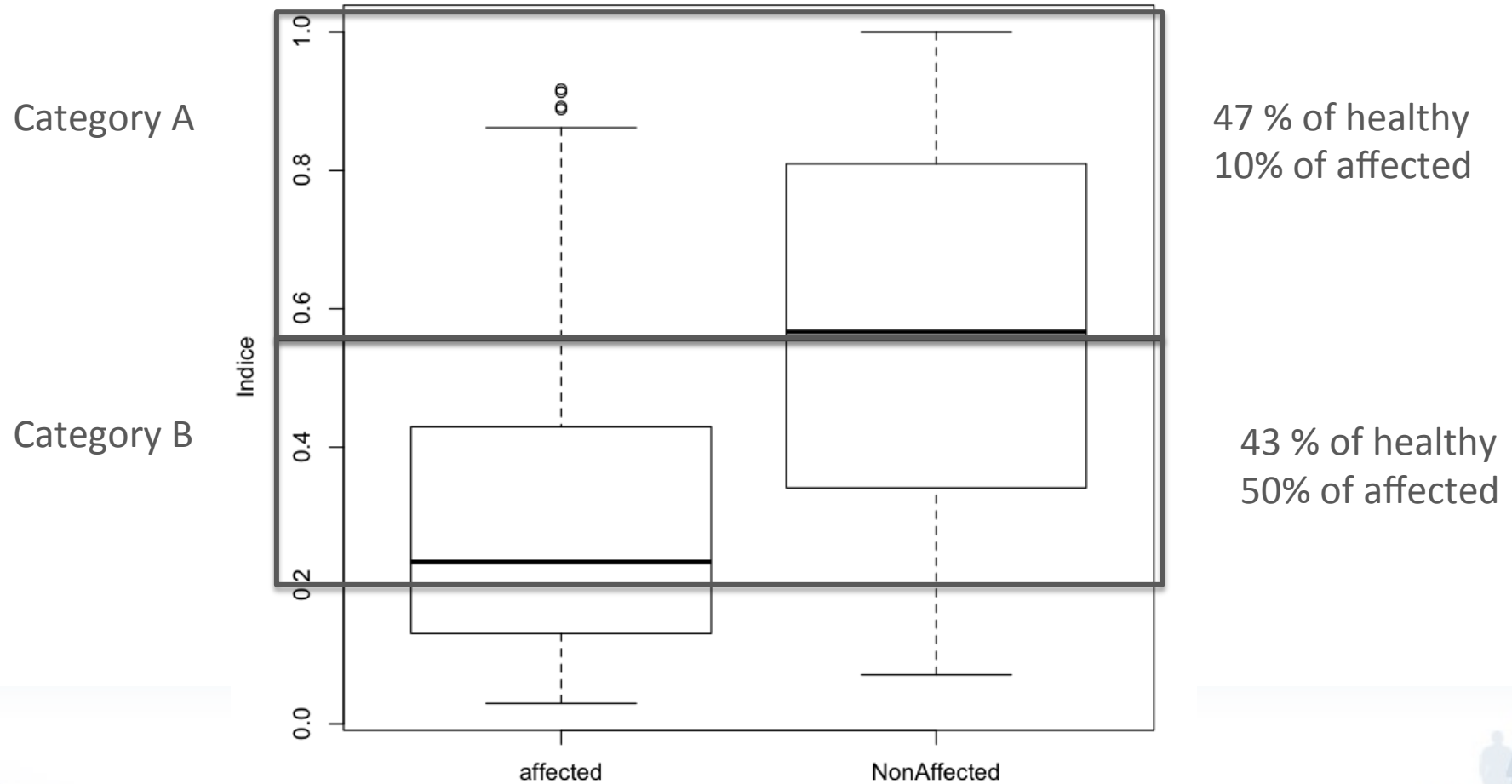Index distribution in case and control populations on 256 affected BMDs and 165 unaffected BMDs

Category A

47 % of healthy
10% of affected

Index development

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

Category A

Category B

47 % of healthy
10% of affected

43 % of healthy
50% of affected

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Index development

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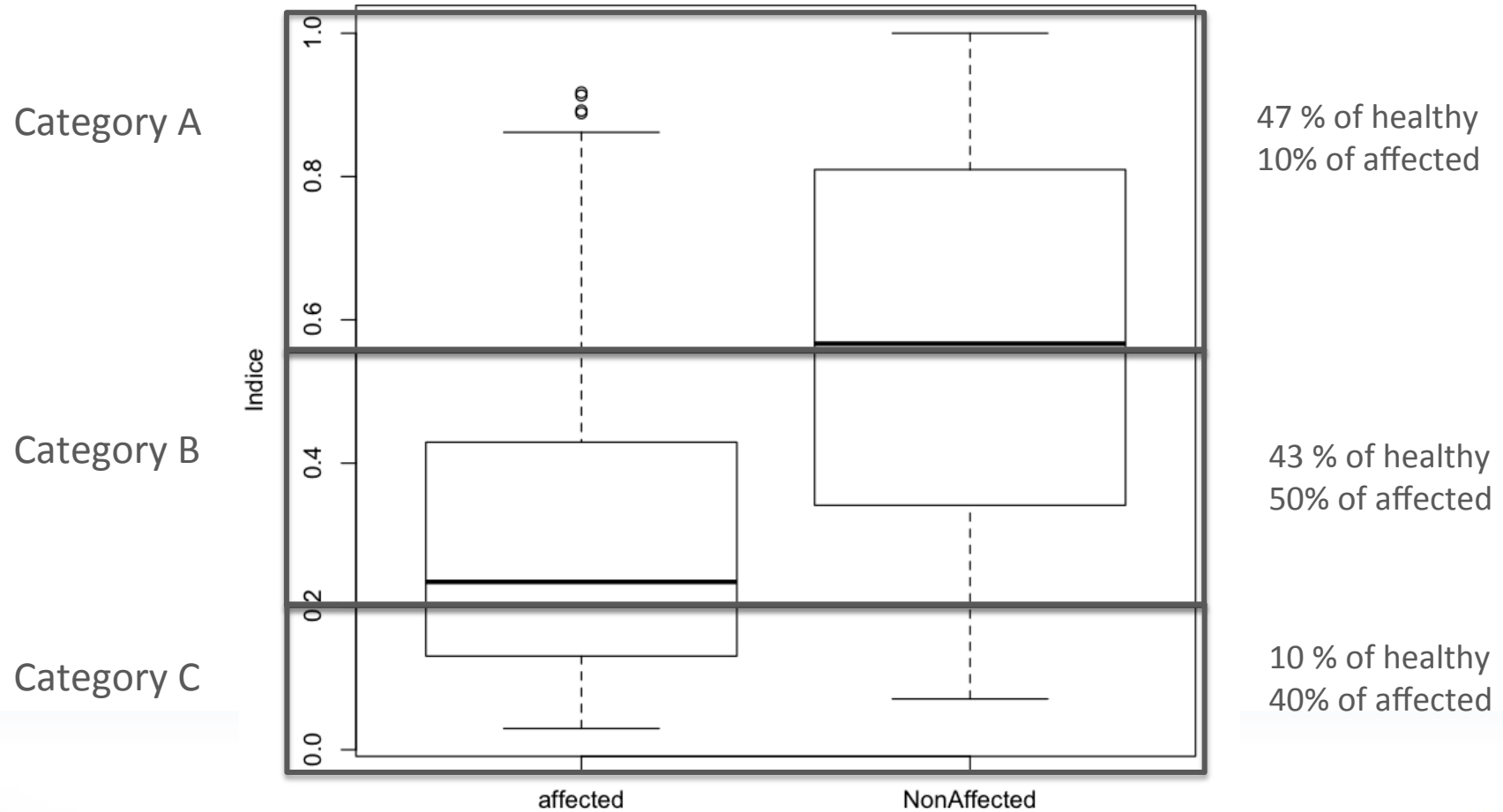

Category A — 47 % of healthy / 10% of affected

Category B — 43 % of healthy / 50% of affected

Category C — 10 % of healthy / 40% of affected

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Validation of Index:
On European population

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**

Validation of Index:
On American population

In collaboration with the 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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	A	B	C
Affected	9.26%	50%	40.74%
Healthy	35.14%	48.65%	16.22%

83%

➢ **Pre test validated for American BMDs :**
   The HS pre-test will be available for American and Canadian dogs as soon as possible

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Summary of Index Validation:

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

➢ 415 dogs with Histiocytic Sarcoma

➢ 303 unaffected dogs (> 10 years)

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

➢ **Statistics :**

88%

Index A to detect healthy dogs :

Positive predictive value ie **probability that a dog A is healthy** : **68.3%**

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

Index C to detect affected dogs :

Positive predictive value ie **probability that a dog C is affected** : **82.8%**

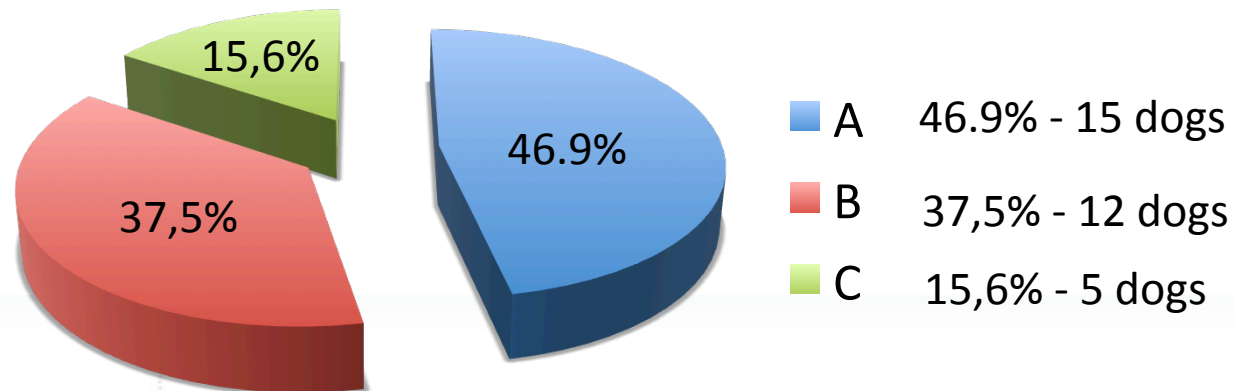Specificity ie **probability that a healthy dog is not C** : **87.8%**

Summary of tests performed by Antagene

Since 2012 , Antagene has tested 757 dogs (unknown phenotypes),
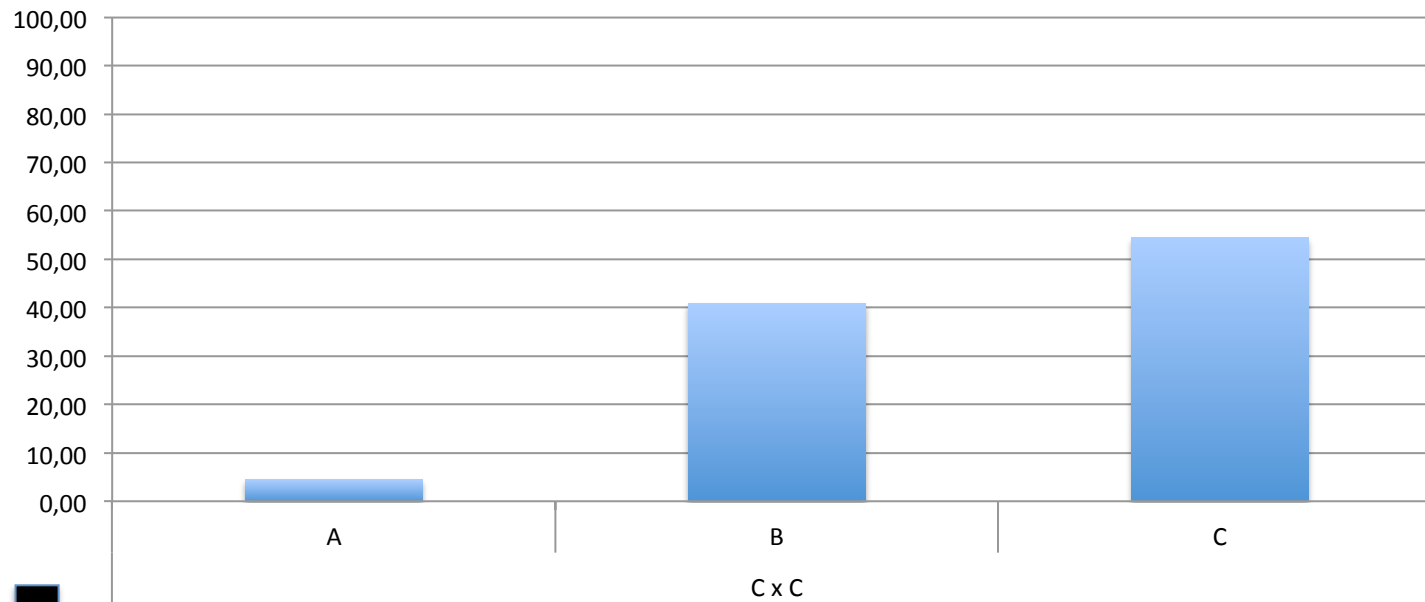
distribution of index in this population:

- A 22.6%
- B 44.4%
- C 33%
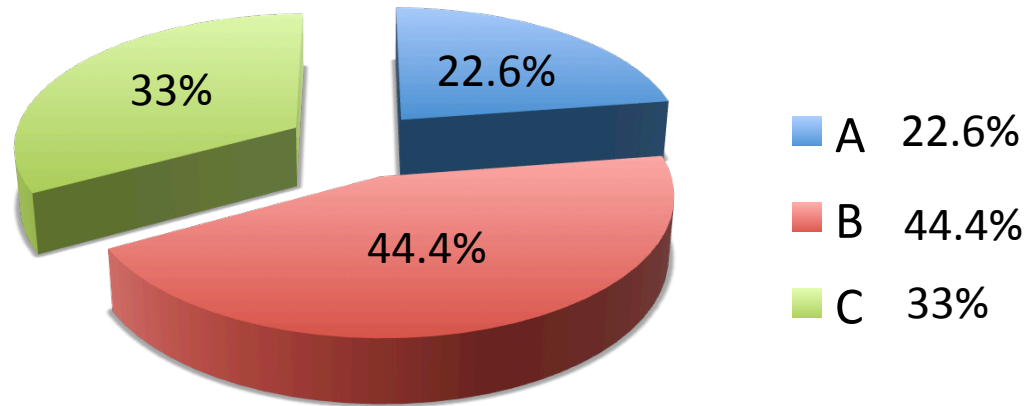
distribution among 32 dogs >10 years old

- A 46.9% - 15 dogs
- B 37,5% - 12 dogs
- C 15,6% - 5 dogs

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

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Study of Index in complete litters

In collaboration with French club (AFBS), Antagene, we tested : 112 puppies from 18 litters:
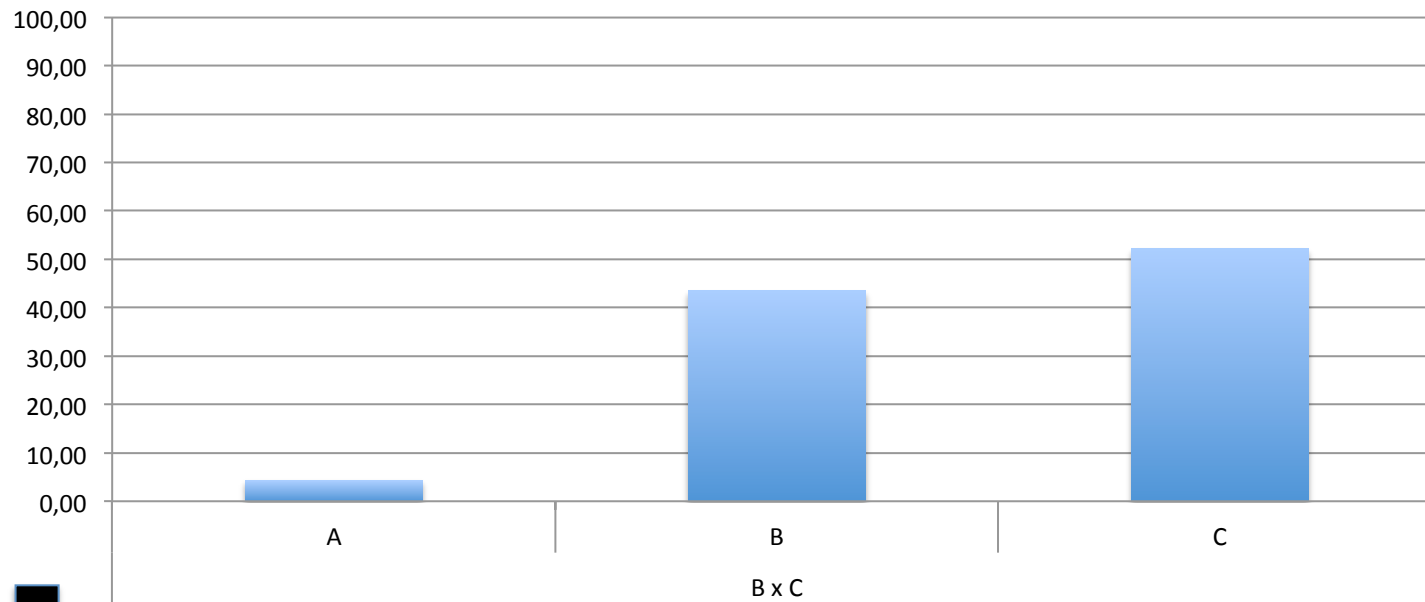
> **Mating : C x C**

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

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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

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Study of Index in complete litters

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

➢ **Mating : A x C**
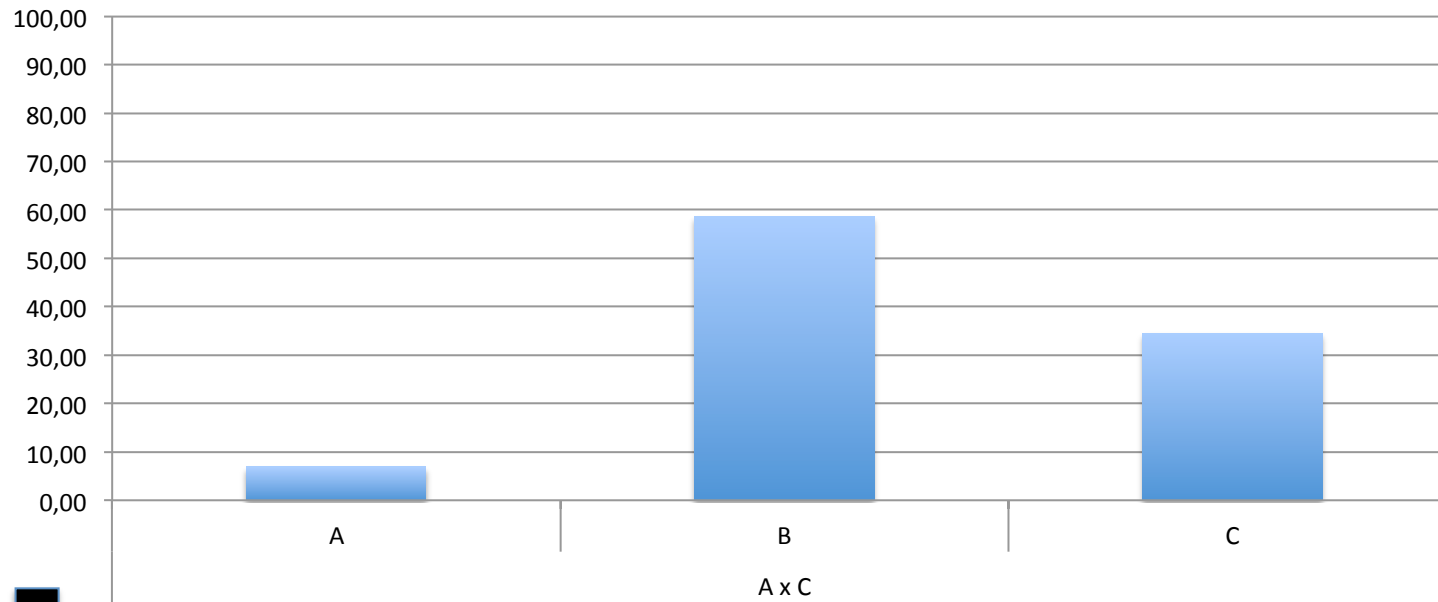
6 litters – 29 dogs
7% of A
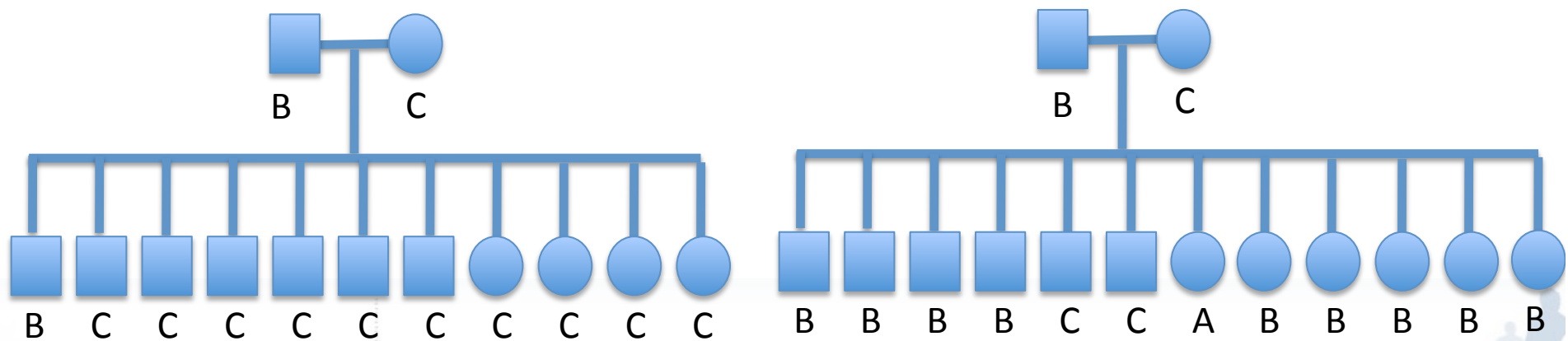**58.5 % of B**
34.5% of C

Summary of the Index study
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.

Compatibility of sire and dam varies!

Summary of the Index study in complete litters

➢ **High variability of index in a blood line or in litters**

➢ **Exceptionally A x A dogs can produce C dogs**

➢ **Exceptionally C x C dogs can produce A dogs**

⮑ **Compatibility of sire and dam varies!**

➢ **Development of a tool HSIMS (Histiocytic Sarcoma Index Mate Selection)
to predict the percentage of A, B and C in a litter (from 2 tested parents)**

Validation on 112 puppies   (expected: 12.5 A,   32.3 B,   23.1 C
    18 litters   observed: 10 A,   32 B,   26 C)

additional tool to adapt your matings more acurately!
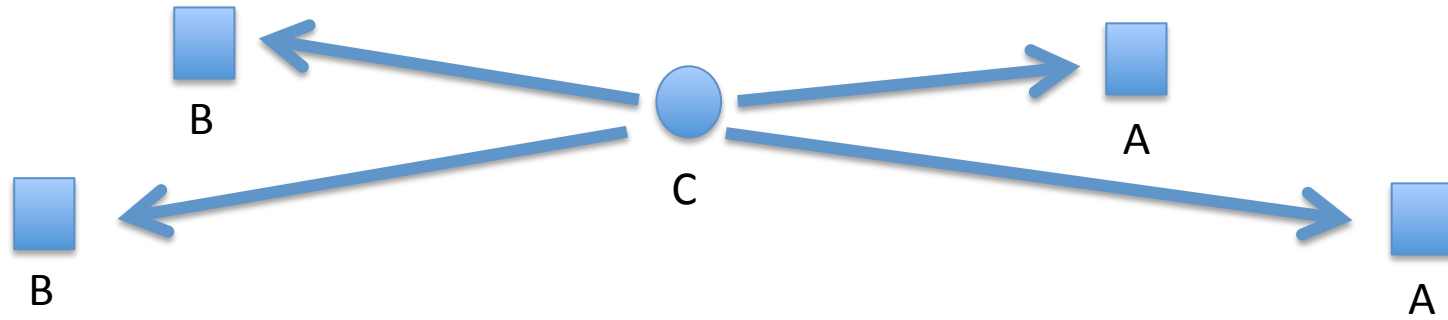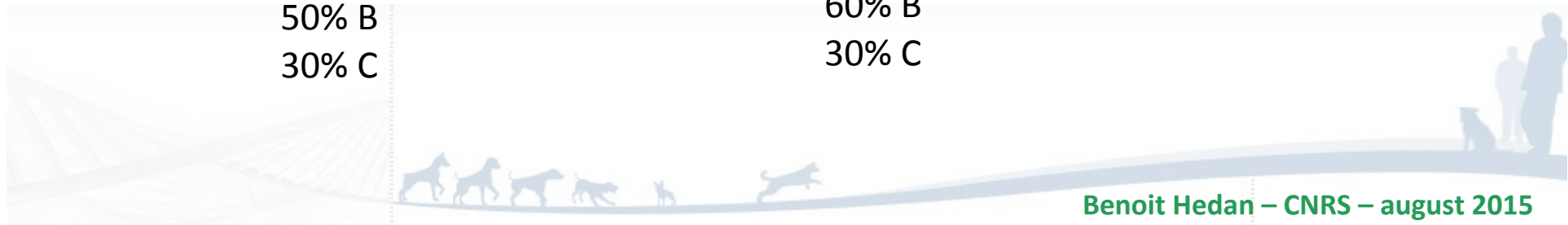
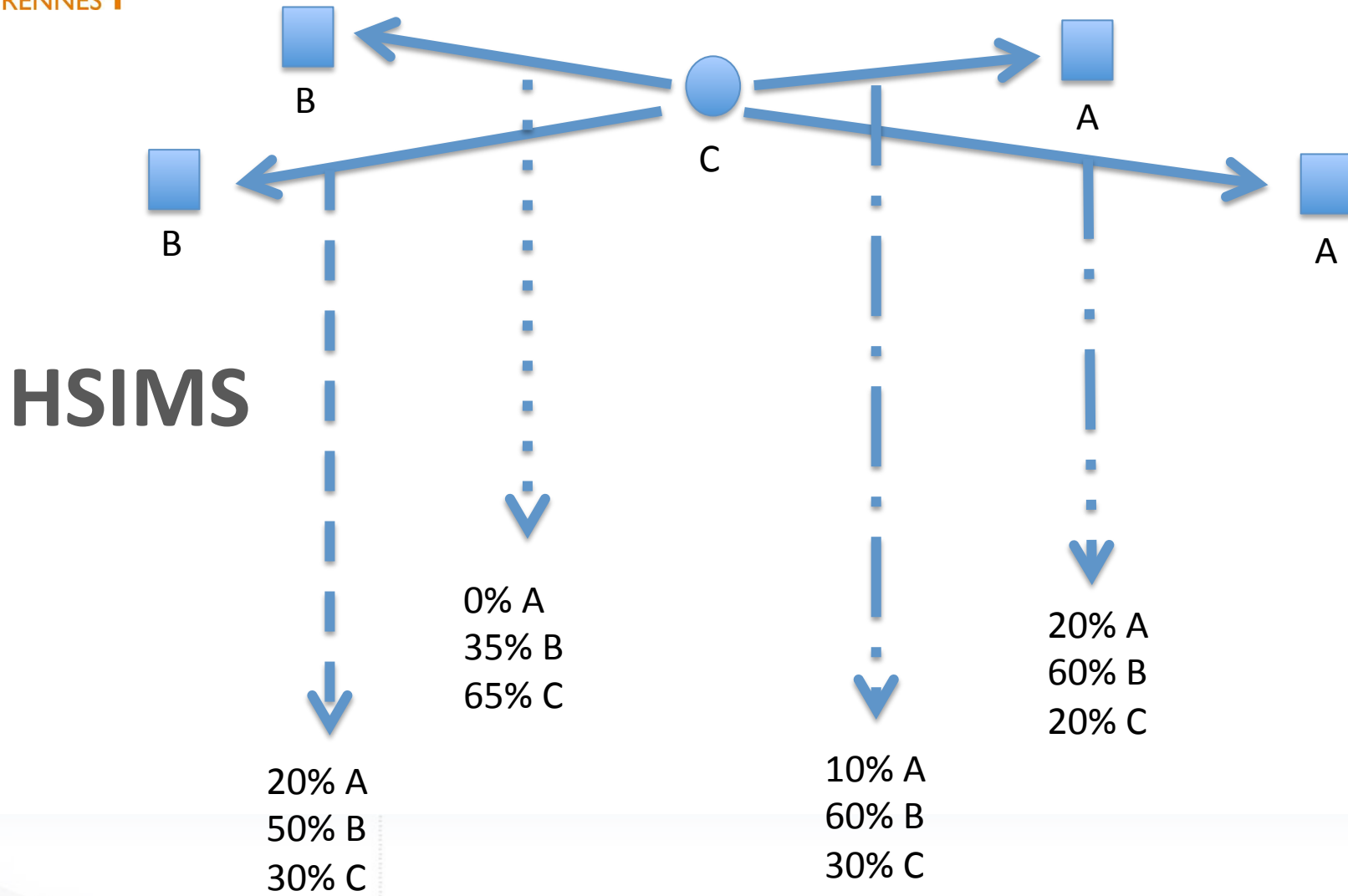Available soon at Antagene

Use of HSIMS :

C

Use of HSIMS :

Use of HSIMS :

HSIMS

B

B

C

A

A

20% A
50% B
30% C

0% A
35% B
65% C

10% A
60% B
30% C

20% A
60% B
20% C

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Use of HSIMS :

HSIMS

0% A
35% B
65% C

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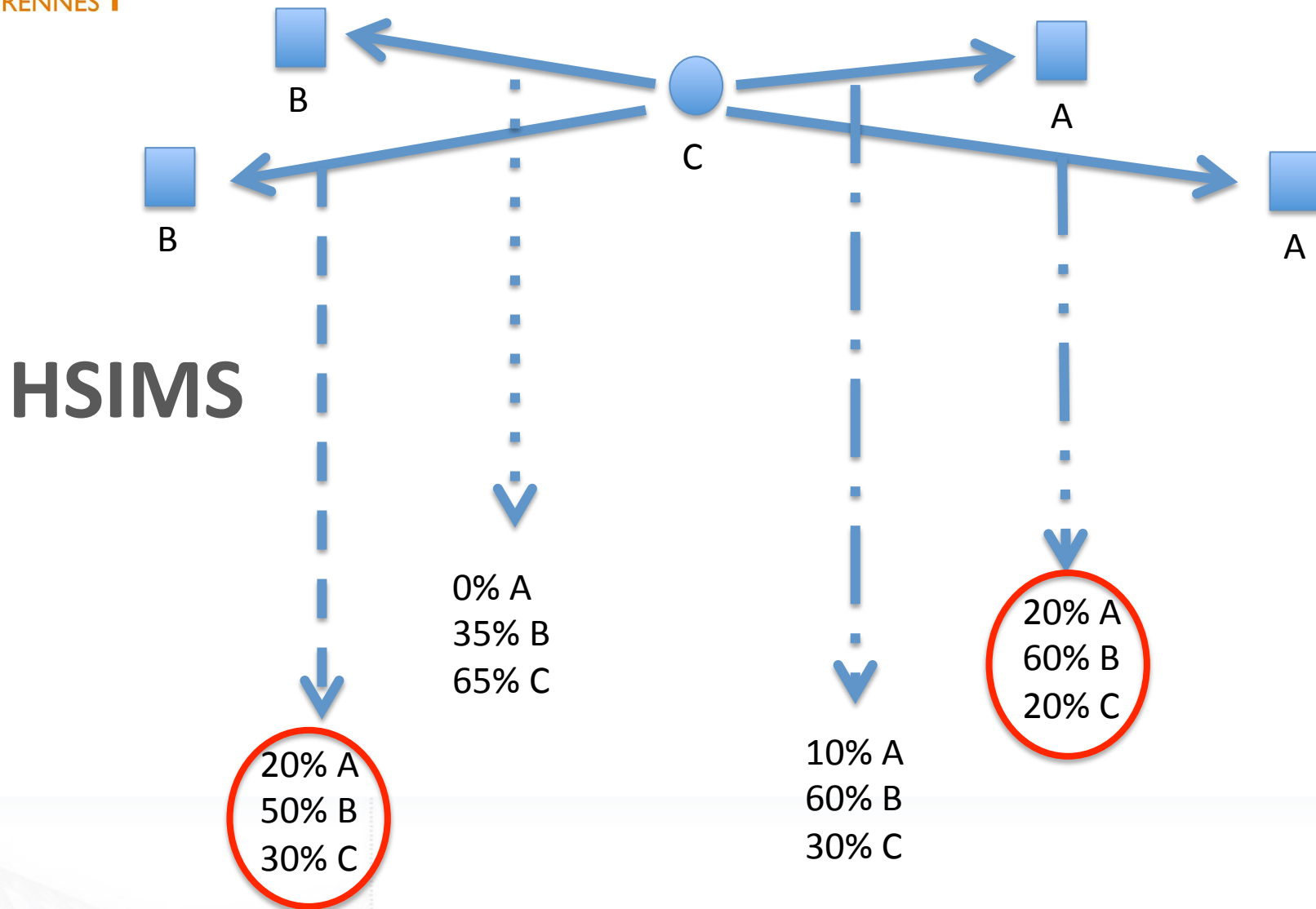
Conclusion and perspectives

➢ **Pre-test**

Validation of the pre-test in different populations
718 dogs from France, Europe and USA :

➔ **pre-test is now available for all BMDs' breeders!**

Observation of specific compatibilities between sires and dams
-> Development of a complementary tool to estimate distribution of index in litters
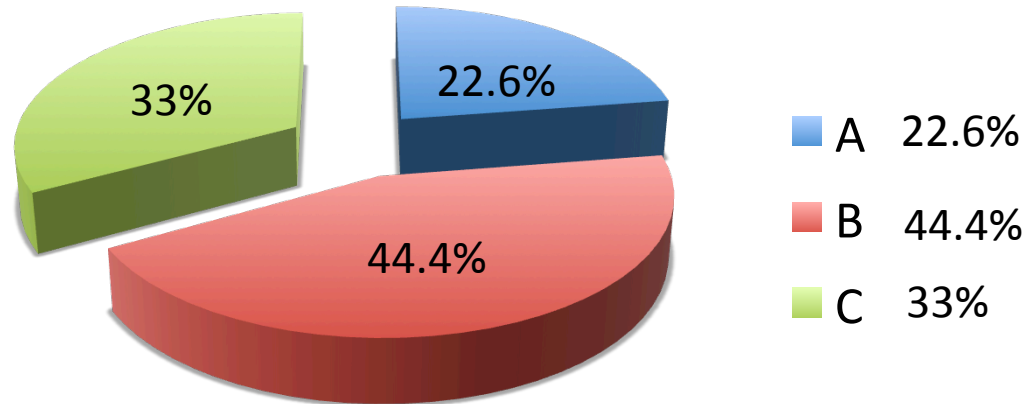
➔**HSIMS : Useful tool for breeders to adapt matings**

➢ **Research is continuing!** ➔ **Samples collection is still needed**

Multigenic and multifactorial cancer (in dog and human HS)

Effort to understand transmission and identify causal mutations

➔ **Will improve the pre-test**

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Take home message

A 22.6%
B 44.4%
C 33%

➢ **You will have to work with C dogs!**

➢ **Keep diversity!**

➢ **This index should be just one of many selection criteria**

Not all dogs at risk (index C) will develop the cancer

Not all dogs with a good index (index A) will be healthy

You will have to work with C dogs!

➔**Mate them with the most compatible A or B !**

Acknowledgments

Canine Genetics Team
Catherine Andre et al.
CNRS Rennes France

The Cancer genetics branch NIH
Elaine Ostrander

NCSU Vet School
Matthew Breen

MSU Vet School
Vilma Yuzbasiyan-Gurkan

Antagene
Anne Thomas
Caroline Dufaure de Citres
Lyon France

French Vet Schools
Jerome Abadie
Patrick Devauchelle

Owners, breeders,

All clubs, Berner Garde,... who sent samples

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