



## WHAT WE DID

- 🐾 Creation of a Bullmastiff database together with information gained from the Australian Bullmastiff Health Survey and owners was used to determine factors affecting longevity, prevalence of diseases, and heritability of lymphoma.
- 🐾 Genetic analysis was conducted using DNA from blood/semen samples from 300 dogs to identify any genomic regions associated with lymphoma.
- 🐾 Diversity within the population we studied was assessed using both pedigree and genetic data.
- 🐾 The frequency of the risk region identified was investigated in an additional 5000 dogs from 130 breeds to assist in validating the region.

## CURRENT STATUS OF PROJECT

A region associated with increased risk of lymphoma in dogs 6 years and younger has been identified and can be screened for. The study itself has reached completion due to the end of Sally's PhD candidature and exhausted funds. Options for continued screening and breeding management are in discussion.

## RESULTS

### Survey data

- 🐾 *Lifespan:* The average age of death reported was <7 years.
- 🐾 *Cause of death:* The most common cause of death reported was lymphoma followed by reports of unknown cancers, osteosarcomas, mammary cancer and brain tumours.
- 🐾 *Type:* The most common form of lymphoma reported was multicentric B-cell lymphoma.
- 🐾 *Age of diagnosis:* Most dogs diagnosed with lymphoma were between 4-6 years of age.

### Genetic Analysis

The following results are based on data from our study population only and may not be fully representative of the entire Bullmastiff population. However, we did capture representative individuals from 70% of popular sires from the last 5 years.

- 🐾 Overall it was found that there is limited diversity, evidence of popular sire use and ancestral inbreeding in the population. Current levels of diversity, however, are not unusual and similar to that of other breeds with comparable registry sizes such as the Dachshund, Samoyed and Fox terrier. Inbreeding levels have stabilized over the last 20 years; however, evidence of popular sire use is still present in the population.
- 🐾 After analysing genetic data from 170,000 points along the DNA of 194 Bullmastiff dogs, including dogs affected by lymphoma, and screening the region of interest in a further 106 dogs, we identified a region in the DNA that is associated with increased risk of lymphoma in dogs 6 years and under. Dogs carrying two copies of the risk region, one from both the dam and sire, have an increased risk of developing lymphoma. When tested additional dogs for the risk "gene" to increase the total, we found that of healthy dogs up to 7 years of age, ~20% have two copies of the risk region and ~55% carry one copy.

### IMPORTANT!!

- 🐾 **A dog with two copies is NOT guaranteed to get the disease, but based on the information we have gathered so far, they are at increased risk. Dogs with 2 copies are 20x more likely to develop lymphoma under the age of 7.**
- 🐾 **It does NOT mean that a dog that only has one or no copies of the region will never get lymphoma. There are multiple risk factors, including age, immune system dysregulation from overstimulation or immunological disorders, and environment that can contribute.**

## WHAT DOES THIS MEANS FOR BREEDING?

Table 1 below shows the outcomes of breeding individuals with two, one and no copies of the risk region. The percentage of puppies expected to have the risk region based off a certain mating is given along with the likelihood of those puppies developing lymphoma. The proportion of puppies with and without the risk region are based off a simple Mendelian inheritance model and do not represent definitive numbers but rather what is expected by chance.

**Table 1. Mating outcomes.** This table describes the predicted outcome of mating dogs with and without the risk region and the predicted risk of lymphoma in offspring.

Dog Status	Two Copies (Risk)	One Copy (Carrier)	No Copies (Clear)
Two Copies (Risk)	100% puppies - two copies  All = 20x more likely to develop lymphoma $\leq$ 6 years	50% puppies - two copies 50% puppies - one copy  Two copies = 20x more likely to develop lymphoma $\leq$ 6 years	100% puppies - one copy
One Copy (Carrier)	50% puppies - two copies 50% puppies - one copy  Two copies = 20x more likely to develop lymphoma $\leq$ 6 years	25% puppies - two copies 50% puppies - one copy 25% puppies - no copies  Two copies = 20x more likely to develop lymphoma $\leq$ 6 years	50% puppies - one copy 50% puppies - no copies
No Copies (Clear)	100% puppies - one copy	50% puppies - one copy 50% puppies - no copies	100% puppies - no copies

## WHAT NOW?

**Implementation Phase:** The success of the project in identifying a risk region for lymphoma means the results can now be used by the breed clubs and breeders to make informed breeding decisions to reduce the incidence of lymphoma in the breed. Funding for the study has ended and as such screening for the region can no longer be provided free of cost. The National Bullmastiff Breed Council (comprising of all member clubs) can decide whether they would like us to continue a screening service for the risk region, however this would incur a cost to cover consumables and labour. The screening test itself is cost effective and simple however the limiting factor is that it has to be conducted in batches of 100 dogs to meet the minimum run costs of the procedure. Fewer dogs can be run in a batch however the same minimum cost applies resulting in an increased cost per dog (estimate below). If continued screening is desired, samples can be sent to the University for processing and storage and when a batch of samples are received they will be screened and results provided to owners. Payment for screening would need to be provided upfront on sample submission. The average turnaround for a full batch would be 3-4 weeks. We understand a test that can be done immediately without having to wait for further samples would be more desirable, but right now a simple test does not exist and would require funds for continued research into the feasibility of a test design and validation. Blood also remains the most reliable source of genetic material. Sally will be finishing at the university in December and as such Associate Professor Peter Williamson will be the primary contact for further research and screening.

### Screening Cost (Approximate estimates):

Batch of 100 = \$50 per dog

Batch of 50 = \$100 per dog

Batch of 25 = \$200 per dog

**Monitoring Phase:** In order to determine the success of any implemented breeding strategies we recommend monitoring the incidence of lymphoma in the breed, and if screening is continued we recommend monitoring the frequency of the risk region in the population.

**Reassess:** We recommend the National Bullmastiff Breed Council (comprising of all member clubs) reassess the need for continued screening or adjustment of breeding recommendations in future based on any changes in the incidence of lymphoma in the breed and frequency of the risk region in the population.

## POTENTIAL BENEFITS

Knowledge of the presence of the risk region in dogs can now influence breeding decisions to reduce the incidence of lymphoma in future litters. This will prevent potential suffering of the progeny while avoiding potential treatment costs and loss of life. This information also means any dogs at risk can be monitored closely for early detection of lymphoma, potentially improving treatment outcomes. Overall it will lead to improvements in health of the breed with the ultimate goal to reduce the frequency of lymphoma.

## FINAL RECOMMENDATIONS

- 🐾 Continued screening would allow breeders to make informed decisions about which animals to breed from to lower the frequency of risk region in the population and lower the lymphoma risk in subsequent generations.
- 🐾 Breeding between two dogs both with two copies of the risk region should be avoided. Breeding a dog with two copies to a carrier (one copy) will also result in a high number of offspring at risk and should be avoided. We also recommend avoiding the mating of two carriers, which will produce an expected 25% of offspring at risk.
- 🐾 As ~50% of the population carry the risk region it is not sustainable for the breed to avoid using these animals altogether as this would threaten the genetic diversity of the breed, increase chances of other inherited diseases and likely result in the loss of desired traits.
- 🐾 Matings between clear animals, or carriers to clear would dilute the frequency of the risk region in the population
- 🐾 A dog with two copies is not guaranteed to get the disease but simply has a much higher risk (approx 20x).
- 🐾 The numbers of dogs in the Bullmastiff population with two copies of the risk region, and those carrying one copy should be monitored along with the incidence of lymphoma. The need for continued screening and should be reassessed in future.

## PUBLICATIONS

Mortlock S-A, Wei J, Williamson P: **T-Cell Activation and Early Gene Response in Dogs**. *Plos One* 2015, **10**(3).

Mortlock S-A, Khatkar MS, Williamson P: **Comparative Analysis of Genome Diversity in Bullmastiff Dogs**. *PLoS ONE* 2016, **11**(1):e0147941.

Mortlock S-A, Booth R, Mazrier H, Khatkar MS, Williamson P: **Visualization of Genome Diversity in German Shepherd Dogs**. *Bioinformatics and Biology Insights* 2016(Supplementary Material 30524):37-42.

Daly, J.-A., Mortlock, S.-A., Taylor, R. M. & Williamson, P: **Cluster Analysis of Tumour Suppressor Genes in Canine Leukocytes Identifies Activation State**. *Bioinformatics and Biology Insights* 2016:59-67.

Mortlock S-A, Khatkar MS, Bennett P, Williamson P: **Lymphoma risk in Bullmastiff dogs is associated with the Myc-PVT1 region on CFA13**. *Submitted*.

## THANK YOU

The Bullmastiff Health Project team at the University of Sydney would like to extend their gratitude to everyone that has supported and participated in the study. Without your participation the success of the project would not have been possible.