Dept. Animal Breeding and Genetics
Swedish University of Agricultural Sciences (SLU)
Uppsala, Sweden

To whom it may concern

A statement concerning the genetic basis for the immune-mediated rheumatic disease and steroid-responsive meningitis arteritis disease complex in Nova Scotia duck tolling retriever dogs.

It has come to our attention that our recent publications concerning the genetic risk factors for the immune-mediated rheumatic disease (IMRD) and steroid-responsive meningitis arteritis (SRMA) disease complex in Nova Scotia duck tolling retriever dogs or "Tollers", has left some uncertainty concerning the genetic basis for the disease complex. We would therefore like to explain our views and to clarify this subject and its implications for breeding. The disease is complex and has many genetic risk factors and therefore ***we cannot provide recommendations for breeders exclusively on the basis of genetic testing for dog leukocyte antigen (DLA) class II genotype.***

1. The inheritance of the IMRD and SRMA disease complex

All our data and all data we are aware of concerning the inheritance of IMRD and SRMA disease complex indicate complex inheritance. This means that there are several genetic factors involved that will influence the disease phenotype. Furthermore, environmental factors will also influence disease status. This means that any given Toller dog has inherited a particular combination of genetic risk factors and that the development of and severity of disease will depend on which combination of the genetic risk factors it has inherited, but also the overall genetic background and the environmental factors that the dog will experience during its lifetime. Importantly, like in other complex diseases, as a consequence of unique environmental exposure the disease aetiology for the disease complex will differ in different Tollers even though they have inherited the same set of genetic risk factors.

It should be noted that our genome-wide association (GWA) study presented in our Nature Genetics paper from 2010 (Wilbe et al. Nature Genetics 42:250-254) was based primarily on a case¬control population of Tollers from Sweden and Finland and validated using Tollers from the US. In these Tollers, significant association was obtained for all five regions. Importantly, some were stronger than others and only two was shared between IMRD and SRMA. The frequency of the actual genetic risk factors may differ in different Toller populations present in different countries. However, they are likely to be similar given the population structure in Tollers and how the breed was created. All of the current Tollers, world-wide, are derived from a small group of dogs that survived two devastating outbreaks in the early 20th century of canine distemper virus epidemics. This resulted in what geneticists call "genetic bottle-necks" and a strong founder effect for the genetic risk factors causing IMRD and SRMA.

The results from the GWA study identified five regions containing many strong candidate genes involved in T-cell activation (Wilbe et al. Nature Genetics 42:250-254). In our Immunogenetics paper from 2009 (Wilbe et al. Immunogenetics 61:557-564) we showed that dog leukocyte antigen (DLA) is another genetic risk factor for development of IMRD. The studies provided conclusive evidence that there are multiple genetic risk factors underlying the IMRD and SRMA disease complex. Importantly, we showed that some of these risk factors were specific for IMRD and that some were common between IMRD and SRMA. The actual mutations causing the disease have not yet been conclusively determined. Intensive research efforts in our laboratory are in progress to identify and validate such mutations. When the mutations have been identified and correlated genetically to disease development genetic tests for all the mutations can be developed.

2. The role of dog leukocyte antigens (DLA) class II in Immune-mediated rheumatic disease The results presented in our Immunogenetics paper from 2009 (Wilbe et al. Immunogenetics 61:557-564) identified one DLA class II type as a genetic risk factor for the immune-mediated rheumatic disease (IMRD) but not for steroid-responsive meningitis arteritis (SRMA). If a dog has inherited the risk DLA

class II type from both parents it likely has an increased risk of developing IMRD. Importantly, the DLA class II type is not the only genetic risk factor, which means that some dogs without this risk factor can still develop IMRD and the opposite is also true. Some individuals with the DLA class II risk type do not develop disease. Our data clearly showed that homozygosity for the risk DLA type is increased among IMRD-affected Tollers. The results from this study were based on a case-control population, which means that we used all our cases and compared to the same number of

healthy dogs. Therefore, neither the frequencies of diseased dogs nor the frequencies of haplotypes do reflect the total frequency in the Toller population. A total of five DLA class II haplotypes was identified and this is similar to most other dog breeds. Furthermore, Hughes et al. (Tissue Antigens 75(6):684-90, 2010) identified two additional haplotypes which gives the Tollers a total of seven known DLA haplotypes. Typically, one or a few DLA types are increased in frequency in any given dog breed. However, we cannot accomplish reduced incidence of IMRD only based on a breeding practice based on DLA genotyping. Inadvertent increase in the frequency of any of the other five known genetic risk factors may be a consequence. We anticipate that when we have DNA tests for all genetic risk factors and knowledge of how they interact we may be able to give potential breeding recommendations how to reduce incidence of the disease.

3. DNA tests and Recommendations for breeding

Commercially available DNA tests for DLA have been offered to Toller breeders since 2010. This DNA test can be used to identify carriers of the DLA risk type in heterozygous or homozygous form. Any DNA laboratory skilled in the art of DNA testing can perform this test and there is no patent protecting its use. Importantly, we have not yet established diagnostic DNA tests for the other five genetic risk factors. The establishment of such tests will require some further research. However, at present testing for DLA only is of limited use.

We cannot provide recommendations for breeders exclusively on the basis of genetic testing for dog leukocyte antigen (DLA) class II genotype. We strongly discourage breeders to perform their dog breeding only on the basis of DLA genotyping. This may lead to increased risk of inheriting unwanted combination of other major genetic risk factors for the disease complex. ***Attempts to reduce the incidence of the IMRD and SRMA disease complex can and should only be based on genotype data on all the genetic risk factors, thereby*** avoiding the most disadvantageous combinations of genetic risk factors. Therefore, there is no current way for breeders to perform DNA testing to reduce or eliminate this disease.

September 12, 2011

Göran Andersson1, Dannika Bannasch2, Helene Hansson-Hamlin3, Kerstin Lindblad-Toh4,5, Hannes Lohi6, Claire Wade7 and Maria Wilbe1

1Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences (SLU), Biomedical centre, Box 597, SE-751 24 Uppsala, Sweden.2Department of Population Health and Reproduction, School of Veterinary Medicine, University of California Davis, Davis, CA3Department of Clinical Sciences, SLU, Box 7054, SE-750 07 Uppsala, Sweden4Department of Medical Biochemistry and Microbiology, Uppsala University, Box 597, SE-751 24 Uppsala, Sweden.5Broad Institute of Harvard and MIT, 7 Cambridge Center, Cambridge, Massachusetts 02142, USA.6Department of Veterinary Biosciences, University of Helsinki, Box 63, 00014 Helsinki, Finland.7Faculty of Veterinary Science, University of Sydney, NSW 2006, Australia