

PROPOSAL FOR CHRONIC HEPATITIS RESEARCH PROJECT

Principle Researcher:
PENNY WATSON MA VetMB CertVR DSAM DipECVIM MRCVS

The Queen's Veterinary School Hospital
University of Cambridge

Summary of Research:

Chronic hepatitis is a relatively common disease in dogs, but with the exception of copper storage disease in Bedlington Terriers, the causes and thus most effective treatments are poorly understood. The English Springer Spaniel (ESS) Clubs of both Great Britain and Norway have noticed a marked increase in chronic hepatitis in the breed in recent years. Many cases look very similar, both clinically and histologically and the appearance suggests a metabolic or infectious cause. The aim of the proposed research project is first to characterise the disease better histopathologically and then look for a cause both via searches for a novel virus (to which ESS may have a breed-related increased susceptibility) and then via pedigree analysis and subsequent genetic analysis of affected and non-affected dogs. The hope is to find the cause of the condition, which will help treatment of affected dogs, and ultimately to develop a genetic test to allow it to be removed from the breed.

Significance of Research:

Chronic hepatitis (CH) is a relatively common disease in dogs and yet the cause is usually unknown¹. Increased incidences of CH in certain breeds have long been recognised but, with the exception of a small number of well-defined copper storage diseases such as those in Bedlington terriers, there have been no recent advances in the understanding of these diseases. Treatment therefore remains non-specific and often not very effective¹. The field of canine genetics has advanced rapidly in recent years²⁻⁵. It is now possible to identify polymorphisms associated with the genetic basis for a disease with an unknown aetiology to allow a positional focus on candidate genes for carriage of the disease associated mutation. This project represents a very exciting and unique opportunity to investigate CH in a pedigree breed of dog via microbiology and genetic analysis with the aim not only of providing a genetic test to allow it to be eliminated from the breed, but also to understand the cause and therefore allow more effective treatment of affected dogs.

Background of Research and Preliminary Work:

Over the last 2 to 4 years, the Health Co-ordinators of the English Springer Spaniel Clubs (UK)¹ noticed an apparent marked increase in reports of a severe form of chronic hepatitis in English Springer Spaniels (ESS) in the UK. It appears to have a high mortality and affects predominantly young to middle-aged female dogs, although males are also affected. They were very concerned and therefore approached Penny Watson at the Department of Veterinary Medicine, University of Cambridge (DVM UC) with a view to co-ordinating a project to find the cause of the condition. The Health Co-ordinators were particularly keen to undertake genetic studies, following the recent success of their genetic investigations of alpha-fucosidosis in the breed, also involving researchers at DVM UC⁶.

The Health Co-ordinator of the Norwegian Spaniel Club², a veterinary surgeon working at the Norwegian Veterinary School, had also noticed a similar disease in ESS in Norway. Interestingly, ESS in the UK and Norway are very closely related genetically.

A meeting was held at the KC in London in October 2005 and again at Crufts in 2006 between the Health Co-ordinators of the English Springer Spaniel Clubs (UK)¹ Penny Watson, The

Health Co-ordinator of the Norwegian Spaniel Club², Ellen Skancke from the Norwegian School of Veterinary Science³, Jeff Sampson from The Kennel Club⁴, and Nick Bexfield (Crufts meeting only) to discuss the disease and plan the project. It should therefore be stressed that the English Springer Spaniel breed clubs, as represented by the UK Health Co-ordinators¹, are very committed to supporting this project as it was in fact started on their initiative. The first result of these meetings was a letter to the Veterinary Record⁷ alerting veterinary surgeons to the disease. Penny Watson has received many emails and phone calls about cases since this letter was published.

The instigators of this study are convinced that there is strong evidence of a distinctive breed-specific hepatitis in ESS, because of the clinical and histological similarities between the cases. Subsequent observations of the histology of cases suggests there may be either a metabolic or a viral cause to which ESS may have an increased susceptibility. However, it is very important to ensure that all cases enrolled in the genetics study have the same phenotype, as far as that is possible, so collation of clinical and histological details is an important first part of the study. Even if most ESS with CH have breed-related disease, there will also be some ESS with CH which have other causes and these should be identified and removed from further analysis as much as possible.

Specific Objectives:

Major funding for a full-scale 3 year study is currently being sought from various sources. An initial pump-priming grant of £500 has been awarded by The Alan Emenev Foundation (TAEF). The specific objectives of this grant are to allow us to 'pump-prime' the study by facilitating collection and storage of blood and liver samples from as many affected dogs as possible and collation of pedigree and history details and histological examination of liver biopsy samples. This preliminary work is currently being undertaken, and the value of the pump-priming grant is that it allows us to continue sample collection before full funding is obtained for the larger project.

Clinical and histological description will help veterinary surgeons and owners to identify cases in the future. The end result of this first year's project we hope to be a clinical paper. We have already submitted an abstract to the European Society of Veterinary Internal Medicine, which has been accepted for presentation at their annual congress in September 2007 (abstract attached).

The aims of the larger project are to build on the samples and information gained using this pump-primer to search for a viral cause of CH in ESS and describe the genetic basis of CH in English Springer spaniels in the UK and Norway. To achieve this, the clinical and histological details of the cases must first be analysed as noted above to ensure as far as possible that all the ESS studied have the phenotype we are interested in and not another, unrelated form of CH.

Brief description of research methods for pump-primer project:

1. **Case recruitment:** continue to liaise with the breed Health Co-ordinators in the UK and Norway^{1,2} and other veterinary surgeons in primary and referral practices to recruit cases. Veterinary surgeons are indeed contacting us on an ongoing basis about new cases. We have contacted the major UK Veterinary pathology laboratories (Finns; Northwestern; Abbey; Animal Health Trust, in addition to the UK Veterinary Schools and referral practices) to alert them of our project and they have all been very helpful indeed in sending us blocks of cases. Finns have also put a note on their histology reports back to vets to alert them about our research. We will continue to liaise with all of the above sources in order to obtain further blocks. To be included in the third year genetics study, affecteds will HAVE to have had liver biopsies taken, either under ultrasound guidance or at laparotomy or post mortem. Most cases already have a biopsy so this is not an onerous requirement.

2. **Description of pathology:** all samples will be stained as outlined above and examined and reported using a standardised method⁸ Any cases with other diseases obvious on histology (e.g. copper of other storage disease, neoplasia or infections) will be excluded from further analysis.
3. **Collection of samples for virology:** to search for novel viruses using molecular techniques, we shall need liver tissue preserved in RNA later rather than formalin from as many affected and control (unaffected) dogs as possible. We shall also collect serum samples from the same dogs to search for anti-bodies to viruses. We shall post out RNA later to interested veterinary practices to allow them to take fresh samples when they biopsy the livers of ESS and other dogs.
4. **Pedigree and DNA Analysis:**
 - a. **Collection of DNA for analysis:** EDTA blood is currently only stored from a small number of dogs, but many are still alive so EDTA blood should be obtained from as many affected dogs as possible, along with pedigree information. New cases are now being reported to us at a rate of two or three per month. We anticipate that this rate will be increased as more veterinary surgeons in the UK become aware of our interest. We will also collect samples from Norway and, if possible, other European countries. There has been no evidence so far that this disease is a problem in the USA. Additional samples will be accumulated during the first 12 months of the project, whilst pathological and pedigree analysis are performed. For the latter, we shall contact reporting veterinarians and breeders in order to establish health status for first and second degree relatives of probands. We shall combine this data with the pedigree information in order to perform heritability estimates. In addition we shall use this period to examine mode of inheritance, to see what is the most appropriate way to analyse our sample collection. In those cases where EDTA Blood is not obtainable, DNA will be extracted if possible from formalin fixed sections. Although this DNA is not suitable for whole genome scans, it will be used for haplotype and candidate gene analysis once a confidence interval is established.
 - b. **Control Samples:** Control blood samples will be obtained from older (> 10 years) ESS with no clinical evidence of previous or current liver disease. Full owner consent will be obtained and the blood samples will be analysed for liver and pancreas enzymes. The aim is to obtain between 50 and 100 control samples.

Expected outcome and applications:

The expected outcome is firstly an accurate and detailed clinical and histological description of CH in ESS in the UK and Norway, then identification of any viral cause of the disease and description of the genetic basis.

These outcomes will allow us both to identify and treat cases more effectively and also to develop a genetic test for the breed society to use to help remove this devastating disease from the breed.

Anticipated problems:

We have the full support of the Breed Clubs so can foresee few obstacles. The most practical problem will be defining 'unaffected' (control) dogs, as we will not be taking liver biopsies from these animals to prove they have not got chronic hepatitis. However, since most affected dogs are young to middle aged, we could define a 'control' ESS as an animal over 10 years of age with normal liver enzymes in the blood.

Reference List:

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Footnotes:

¹Lesley Bloomfield and Louise Scott Health Co-ordinators English Springer Spaniel Clubs (UK)

²Wenche Farstad, Professor, DVM ,PhD Health Co-ordinator, Norwegian Spaniel Club.
Norwegian School of Veterinary Science, P.O.Box 8146 Dep, N-0033 Oslo, Norway

³Ellen Skancke, DVM, PhD, Associate Professor Norwegian School of Veterinary Science,
P.O.Box 8146 Dep, N-0033 Oslo, Norway

⁴Jeff Sampson, Kennel Club Canine Genetics Co-ordinator (UK)