

Progress report on the evaluation of the accuracy of anti-neutrophilic cytoplasmic auto-antibodies (pANCA) for early detection of PLE/PLN in the population of Soft Coated Wheaten Terriers in the United Kingdom

In October 2009, Anna Karagianni has joined the RVC team from Greece. We are very lucky in that Anna has decided to do her Master by Research degree at the RVC. Her project will be to organize the pANCA sessions, perform the immunofluorescence assays for determination of pANCA and to finalize and communicate the data from the longitudinal study. You will be able to meet Anna at the next sampling sessions to be organized in February 2010 in case you decide to participate.

Report on sampling 4 of the longitudinal study

Protein Losing Enteropathy (PLE) and Protein losing Nephropathy (PLN) in Soft Coated Wheaten Terriers (SCWT) are two syndromes which are characterised by loss of plasma proteins from the small intestinal gut and the kidney, respectively. Despite the unknown aetiology of PLE/PLN, it is believed that there is a familial inheritance of these diseases in SCWTs. It has been reported that these syndromes affect middle aged to older dogs (4-6 years old) with a slight predilection to females. The main symptoms of PLE/PLN are small intestinal diarrhoea, vomiting, ascites, weight loss and peripheral oedema. The prognosis of PLE and PLN is very poor as the median survival time is only months after the disease is diagnosed.

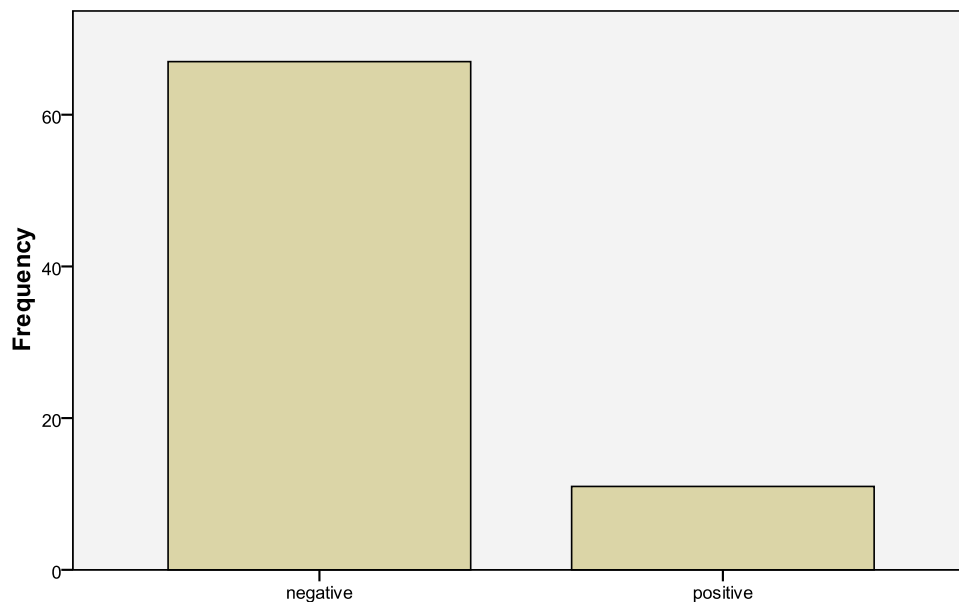
In one study in 2008, Karin Allenspach and colleagues have demonstrated that perinuclear antineutrophilic cytoplasmic autoantibodies (pANCA) may be used as an early clinical serological marker for PLE/PLN in this breed. In this study, it was reported that pANCA occur in SCWTs 2-3 years before the onset of the disease and that the occurrence of pANCA in affected animals is strongly associated with hypoalbuminaemia. Early diagnosis and prevention of PLE and PLN is of major importance for the breed of SCWTs because we may be able to eliminate the disease from this breed while it is not very prevalent yet. Owners and veterinarians will have the chance to prevent the disease and to secure welfare for this breed, as they can take measures (proper nutrition, medication) after the detection of pANCA and before the disease becomes overt.

Since 2007 we have been collecting blood samples of healthy SCWTs and information on their health status. Their pANCA status as well as serum albumin concentrations was determined in all dogs enrolled into a longitudinal study, every six months. In 2007, 39 of 188 healthy tested dogs (20.7%) were found to be pANCA positive. After starting the longitudinal study and enrolling 90 dogs in 2008, 9 of 89 dogs (10.1%) were found to be pANCA positive. Last year, we retested 94 dogs from which 15 (15.9%) were found positive in the first sampling and 11 of 78 (14.1%) in the second sampling.

Table 1: Number of dogs sampled and tested for the fourth sampling of the longitudinal pANCA study in September 2009

Date	Location	Nr. of dogs tested
19/09/2009	Saint Michael's Hall, Lechlade Road	21
26/09/2009	The Village Hall, West Street	30
Diverse	Sent to Royal Veterinary College per post	29
Diverse	Sample taken at RVC	6
<i>Total</i>		78

Table 2: Frequencies of pANCA positive and pANCA negative blood samples tested in the fourth sampling of the longitudinal pANCA study in September 2009



pANCA negative:67, pANCA positive:11, Total number of tested dogs: 78

For the measurement of pANCA, we use a method called Indirect Immunofluorescence. After sample collection, blood samples are placed on ice and transferred to the lab and are kept frozen at -20 until they are analysed. In the last sampling 7 dogs out of 78 have converted from positive to negative or from negative to positive. The reason for this is unknown but it seems to be a physiological phenomenon and has been reported in other studies looking at pANCA immunofluorescence in dogs. It is possible that these dogs have very little antibody in their blood and so, their detection can be just positive or just negative depending on the individual serum level. To determine whether the dogs remain positive or negative over a period of 2 years, it is essential to re-test all dogs included in the longitudinal study.

Albumin is synthesised in the liver and its role is very important for the dog's health as it maintains the osmotic pressure in the body fluids and it carries essential hormones and fatty acids. Low levels of albumin can indicate protein losing diseases including PLE/PLN. So, it is very important for us to measure albumin concentrations of the tested dogs regularly. The reference range for albumin in our laboratory is 28-39g/l. In the 4th sampling of the longitudinal study in September 2009, 62 of the 78 tested dogs (79%) had serum albumin results within the reference range, 2 (3%) were below the reference range and 9 (12%) were above the reference range. The two which were below the reference range had albumin levels of 27 g/l and 26.6g/l which is only borderline low and therefore

not clinically significant. Furthermore, dehydration can increase the albumin concentration in the blood and this may explain many of the high results we saw in healthy SCWT over the last 2 years.

The laboratory findings have been communicated by mail to the owners in keeping with our confidentiality promise. In case you have not received your results yet, please do not hesitate to contact the Clinical Investigations Center (01707 666 605 or 01707 666 223, cic@rvc.ac.uk) at the RVC. Additionally if you would like to discuss the test results of your dogs or have any questions regarding the study or if your dog develops any clinical signs indicative of possible PLE/PLN please do not hesitate to contact Anna Karagianni (akaragianni@rvc.ac.uk) or Karin Allenspach (kallenspach@rvc.ac.uk or 01707 666 366).

By the end of the study in September 2010, we will be able to definitely determine whether pANCA are a useful early test for PLE/PLN in the breed of SCWT. Regardless of the results of the study though, we will have gathered a lot of information regarding general health and pedigree information on healthy and affected dogs. These data together with the archived blood samples we have from so many dogs may well serve for further genetic studies to take place. These studies will eventually allow us to find a genetic marker for the disease and therefore offers the possibility to eradicate the disease in the UK.

As it is very important for the study to gather as much information as possible about the dogs, we would like to encourage all of you to attend the next 2 sample sessions in 2010 even if you have missed any previous ones.

The study will finish in September 2010 when we will communicate the results to the owners, WHI and the SCWT Club of GB.

We would like to thank you very much for participating in this study and wish you a happy Christmas!

Best Regards

Anna Karagianni and the RVC team

Royal Veterinary College