Prevalence of perinuclear antineutrophilic cytoplasmic autoantibodies in serum of healthy Soft Coated Wheaten Terriers in the United Kingdom

Barbara Wieland, Dr med vet, PhD; Jennifer F. Summers, BVetMed, MSc; Barbara Häsler, Dr med vet, PhD; Carolina Mancho-Alonso, DVM, PhD; Amanda Craig, BVSc, MVetClinStud; Karin Allenspach, Dr med vet, PhD

Objective—To estimate the prevalence of perinuclear antineutrophilic cytoplasmic autoantibodies (pANCA) in the serum of healthy Soft Coated Wheaten Terriers (SCWTs) in the United Kingdom and to identify potential risk factors and heritability patterns associated with a positive result for pANCA.

Animals—188 SCWTs (age range, 18 months to 14.3 years).

Procedures—Blood samples were obtained from SCWTs in various locations in England. Serum was tested for pANCA by use of an immunofluorescence assay, and total protein and albumin concentrations were determined. Pedigrees were evaluated to identify close relatives that had protein-losing enteropathy (PLE) or protein-losing nephropathy (PLN).

Results—39 of 188 (20.7%) dogs, including young dogs, had positive results for pANCA. Dogs had significantly higher odds of having positive results for pANCA if they had at least 1 littermate that had PLE or PLN (odds ratio, 12.1) or if they had at least 1 full sibling from another litter known to be affected with PLE or PLN (odds ratio, 4.0).

Conclusions and Clinical Relevance—This study revealed a high prevalence of pANCA in the serum of a representative sample of healthy SCWTs in the United Kingdom and a significant association between positive results for pANCA and a diagnosis of PLE or PLN in a sibling. (*Am J Vet Res* 2012;73:404–408)

Perinuclear antineutrophilic cytoplasmic autoantibodies are a group of autoantibodies that are primarily associated with intestinal inflammation, and they are used as serologic markers of IBD in humans.[1,2] These pANCA are more specific serologic markers of IBD than are other biomarkers (eg, serum C-reactive protein concentration) that indicate nonspecific inflammation. In the field of veterinary medicine, pANCA was first investigated as a serologic biomarker of IBD in dogs.[3] There is a significant association between a positive result for pANCA and a diagnosis of IBD in dogs.[3] Results of a study[4] in a breeding colony of SCWTs that had PLE or PLN (or both) suggest that pANCA could be an early marker of PLE and PLN in SCWTs. The objective of that study[4] was to correlate the pANCA status (ie, positive or negative results for pANCA) of dogs (13 SCWTs and 8 SCWT-Beagle crossbred dogs) with clinicopathologic markers of PLE and PLN. The results of that study also indicated a significant ($P = 0.001$) association between positive results for pANCA and hypoalbuminemia. Twenty of 21 (95.2%) dogs in that study[4] had positive results for pANCA when tested ≥ 2 times, and 18 of 21 (85.7%) dogs developed PLE or PLN (or both) that was diagnosed via clinical or postmortem ex-

Abbreviations

CI	Confidence interval
CMA	Common male ancestor
IBD	Inflammatory bowel disease
pANCA	Perinuclear antineutrophilic cytoplasmic autoantibodies
PLE	Protein-losing enteropathy
PLN	Protein-losing nephropathy
SCWT	Soft Coated Wheaten Terrier

Received March 30, 2010.
Accepted December 22, 2010.
From the Department of Veterinary Clinical Sciences, Royal Veterinary College, University of London, North Mymms, Hatfield, Hertfordshire, AL9 7TA, England (Wieland, Summers, Häsler, Craig, Allenspach); and the Department of Animal Medicine and Surgery, College of Veterinary Medicine, Complutense University of Madrid, Madrid, Spain (Mancho-Alonso). Dr. Craig's present address is Discipline of Pharmacy, Faculty of Health, University of Canberra, Canberra, ACT 2601, Australia.
Supported by the UK Kennel Club Charitable Trust, the SCWT Club of Great Britain, and the Wheaten Health Initiative.
Presented in part as an oral presentation at the 18th European College of Veterinary Internal Medicine Conference, Ghent, Belgium, September 2008.
The authors thank Sandra Jeffries for assistance with collection of data from pedigree records.
Address correspondence to Dr. Wieland (bwieland@rvc.ac.uk).

Unauthenticated | Downloaded 12/04/22 02:59 AM UTC

amination. The serum pANCA assay had a sensitivity of 0.95 (95% CI, 0.72 to 1.00) and a specificity of 0.80 (95% CI, 0.51 to 0.95) for prediction of development of disease (PLE or PLN) in the dogs.

In the United States, PLE and PLN have increasingly become problems in SCWTs since the 1980s. A study[5] that included 222 SCWTs with PLE or PLN (or both) revealed that the onset of PLE and PLN is related to age and sex of dogs. Furthermore, all dogs included in that study[5] were related to 1 CMA, which suggests that PLE and PLN have a familial pattern of inheritance in SCWTs. The first report[6] of PLE or PLN in a SCWT in the United Kingdom was published in 2004. Since that time, the SCWT Club of Great Britain has maintained a databank to which SCWTs that have PLE or PLN (ie, suspicion of PLE or PLN based on clinical examination findings or diagnosis of PLE or PLN based on histologic examination of intestinal or renal biopsy specimens or results of postmortem examination) can be voluntarily reported. Histologic analysis of intestinal biopsy specimens of SCWTs that are affected with PLE reveals signs of intestinal inflammation, including inflammatory cell infiltration of the mucosa,[6] which may be attributable to an immune-mediated process. It has been suggested that hypersensitivity reactions to food may have a role in the development of PLE in SCWTs.[7,8]

In 1 study,[3] the sensitivity of pANCA status to identify IBD-affected dogs was low, but good specificity was reported. Results of another study[4] conducted to investigate a research colony of SCWTs suggested that positive results for pANCA may be a suitable predictor for development of PLE and PLN. However, the prevalence of pANCA in SCWTs and the importance of pANCA as a serologic marker for immune-mediated diseases in dogs have not been investigated. Therefore, the purpose of the study reported here was to estimate the prevalence of pANCA in the SCWT population in the United Kingdom, to identify potential risk factors and heritability patterns for PLE and PLN in SCWTs, and to determine baseline data that may be used in future studies to investigate the use of pANCA as an early serologic marker of immune-mediated diseases in SCWTs.

Materials and Methods

Animals—On the basis of records from the SCWT Club of Great Britain, it was estimated that there are approximately 3,000 SCWTs in the United Kingdom. To confirm an estimated prevalence of 5% of positive results for pANCA in those SCWTs, a sample size of 200 was calculated by use of software.[a] The minimum age of dogs included in the present study was 18 months, and the study population was allocated into 2 groups (young dogs [18 months to 4 years old] and old dogs [> 4 years old]). Recruitment of dogs for this study was accomplished with the help of the SCWT Club of Great Britain and the Wheaten Health Initiative. To ensure wide geographic representation, blood samples were obtained by the investigators from SCWTs in various locations in England during 5 sessions from June through November 2007 or by private practice veterinarians in England during this same period. This method was intended to ensure that a random sample of dogs from various breeding lines was included. The study protocol was approved by the Ethics Committee of the Royal Veterinary College. All owners signed a form consenting to participation of their dogs in the study.

Procedures and immunofluorescence testing—For each dog, a questionnaire was completed by the owner at the time of blood sample collection to determine potential risk factors for PLE and PLN. The information that was collected for each dog included diet; sex; age; history of PLE or PLN in the sire, dam, siblings, and offspring; previous drug and health history; and pedigree. Owners were asked to identify dogs in the pedigree that had heritable diseases and to identify those diseases.

During the blood sample collection sessions conducted by the investigators, each dog was weighed and examined by an experienced veterinarian (KA or AC). For each dog, a blood sample (3 mL) was collected from one of the jugular veins into a tube that contained heparin. Within 3 hours after collection, blood samples were centrifuged at 450 × *g* for 20 minutes to obtain serum, and harvested serum was stored at –20°C until further use. Blood samples collected by private practice veterinarians were prepared this same way and serum was shipped on ice to the Royal Veterinary College within 24 hours after collection.

Detection of pANCA in serum samples was performed by use of an indirect immunofluorescence assay as previously described.[3] Briefly, slides of canine granulocytes were incubated for 1 hour with serum (1:10 dilution) obtained from the dogs. Cells were washed, a secondary fluorescein isothiocyanate–labeled antibody (sheep anti-canine IgG,[b] 1:50 dilution) was added, and slides were incubated for 1 hour in a humid chamber at room temperature (22°C). After a final wash, slides were dried and mounted with fluorescence mounting medium. Slides were evaluated separately by use of fluorescence microscopy by 2 authors (KA and CM) who were unaware of the source of the samples. Sera from dogs that resulted in a specific pattern of perinuclear staining in granulocytes were considered to have positive results for pANCA, whereas sera with no staining or an atypical staining pattern of granulocytes (including evidence of intranuclear staining) were considered to have negative results for pANCA. Serum total protein and albumin concentrations were determined for all serum samples by personnel in the clinical pathology laboratory of the Royal Veterinary College.

Analysis of heritability patterns—Pedigree data were used to calculate 3 indices of inbreeding by use of software[c] at the 8-, 16-, 24-, and 32-generation level for each dog. All 3 indices were based on the position and number of common ancestors in a pedigree and determined the probability that each dog had 2 identical alleles from common descent (coefficient of inbreeding index), the loss of heterozygosity per generation (loss of heterozygosity index), and the amount of heterozygosity that remained in each generation (rate of inbreeding index). A study[5] in the United States identified 1 CMA in 188 of 222 PLE-affected dogs (referred to as the Littman CMA). To assess the importance of the Littman CMA in the United Kingdom, its potential ancestral influence was determined at the 8th and 11th genera-

Unauthenticated | Downloaded 12/04/22 02:59 AM UTC

tion level by use of software.[c] The number of close relatives (offspring, full siblings, sire, dam, grandsires, and granddams) that were affected with PLE or PLN (on the basis of clinical signs and results of histologic examination of tissue samples) of each dog in the present study was determined from the pedigrees. Additionally, the number of ancestors in the 4-generation pedigree that had generated offspring with PLE or PLN was determined for each dog.

Statistical analysis—Statistical analyses were performed by use of statistical software.[d,e] Normal distribution of data was determined by use of the Kolmogorov-Smirnoff test. For each group of dogs that was compared, equal variance of data was determined by use of the Levene test for equality of variances. Group comparisons were performed by use of a *t* test. Univariable logistic regression and the Pearson correlation coefficient were used to test for associations among risk factors in dogs with a positive result for pANCA. Values of $P < 0.05$ were considered significant.

Results

Animals—From June through November 2007, blood samples were collected from 205 dogs, of which 17 had incomplete data and were excluded from the study and 188 had complete pedigree records and questionnaire data and were included in the study. Of the included blood samples, 184 were collected in 1 of the 5 blood sample collection sessions and 4 were collected by veterinarians in private practice. Of the 188 dogs that had complete data, there were 72 male and 116 female dogs, and the mean age was 5.9 years (median, 5.1 years; range, 1.5 to 14.3 years). There were 63 dogs (22 male and 41 female) in the young dogs group and 125 dogs (50 male and 75 female) in the old dogs group. Twenty-four of the dogs included in the study had received medical treatment within 4 weeks before the blood samples were collected. Medication was most commonly administered because of routine deworming or for treatment of minor medical conditions such as an abscess or an ear infection. At the time of blood sample collection, the dogs were typically healthy; clinical examination revealed a few dogs with dental disease or subcutaneous masses. None of the dogs had any clinical signs of PLE or PLN.

Of the 188 dogs, 39 had positive results for pANCA (prevalence of pANCA, 20.7%; 95% CI, 15.1% to 26.4%), and 149 had negative results for pANCA. There was no significant association between pANCA status and sex ($P = 0.69$) or age group ($P = 0.43$) of the dogs. There was no significant ($P = 0.14$) difference in the mean ± SD age of dogs that had positive results for pANCA (6.5 ± 3.5 years; range, 1.6 to 13.9 years) and dogs that had negative results for pANCA (5.7 ± 2.9 years; range, 1.6 to 14.3 years).

Serum albumin concentration (reference range, 28 to 39 mg/dL) was within reference limits in 146 dogs, was high (range, 39.1 to 71.2 mg/dL) in 36 dogs, and was mildly low (range, 26.3 to 27.9 mg/dL) in 6 dogs. Total protein concentration (reference range, 56 to 83 mg/dL) ranged from 66.3 to 113.7 mg/dL. Mean ± SD serum albumin concentration was not significantly ($P = 0.90$) different between dogs positive for pANCA (40.4 ± 9.1 mg/dL) and dogs negative for pANCA (35.7 ± 6.8 mg/dL). There was no significant association between pANCA status and the administration of any medical treatment within 4 weeks prior to blood sample collection (odds ratio, 2.0; 95% CI, 0.8 to 5.1; $P = 0.14$) or between pANCA status and the feeding of a predominantly raw meat diet (odds ratio, 2.0; 95% CI, 0.8 to 4.9; $P = 0.12$).

Heritability variables—No significant differences were detected between dogs that had positive results for pANCA and dogs that had negative results for pANCA for the coefficient of inbreeding index ($P = 0.68$), loss of heterozygosity index ($P = 0.87$), or rate of inbreeding index ($P = 0.87$). The influence of the Littman CMA was similar in dogs with positive results for pANCA and dogs with negative results for pANCA (**Table 1**). Dogs had significantly higher odds of having positive results for pANCA if they had at least 1 littermate that was known to have PLE or PLN (odds ratio, 12.1; 95% CI, 1.2 to 120.4; $P = 0.03$) or if they had at least 1 full sibling (same dam and sire but different litter) that was known to have PLE or PLN (odds ratio, 4.0; 95% CI; 1.1 to 17.2; $P = 0.04$).

Discussion

The objective of the study reported here was to estimate the prevalence of pANCA in SCWTs in the United Kingdom and to identify potential risk factors and heritability patterns associated with a positive result for pANCA. The prevalence of pANCA in the study population was higher than expected. The high prevalence of pANCA in this group of healthy dogs suggests that

Table 1—Appearance of the Littman CMA in pedigrees at the level of the 8th and 11th generations and mean ± SD potential ancestral influence of this CMA in SCWTs that had positive or negative results for pANCA.

Variable	pANCA positive (n = 39)	pANCA negative (n = 149)	Total (n = 188)
CMA at 8th generation at least once (No. [%])	36 (92.3)	139 (93.3)	175 (93.1)
CMA at 11th generation at least once (No. [%])	37 (94.9)	140 (94.0)	177 (94.1)
Potential ancestral influence of CMA at 8th generation (%)	6.8 ± 5.1	6.8 ± 4.3	6.8 ± 4.4
Potential ancestral influence of CMA at 11th generation (%)	9.8 ± 6.0	10.1 ± 5.3	10.0 ± 5.4

No significant ($P < 0.05$) differences were found between pANCA-positive and pANCA-negative SCWTs for any of the variables.
n = No. of dogs.

Unauthenticated | Downloaded 12/04/22 02:59 AM UTC

these dogs could develop immune-mediated diseases later in life. Given that pANCA is associated with IBD in other breeds[3] and there were higher odds of a dog having positive results for pANCA in the present study if it had siblings that had PLE or PLN, these findings may also indicate that pANCA could be a marker for immune-mediated diseases. However, long-term monitoring of the dogs evaluated in the present study would be necessary to confirm whether pANCA is indeed a marker for PLE or PLN.

A positive result for pANCA may be attributable to diseases other than PLE or PLN or to administration of medication. However, there was no significant association between pANCA status and sex, age, diet, or administration of medication within 4 weeks prior to blood sample collection in the present study. Although the finding was not significant, the increased odds for positive results for pANCA in dogs that had raw meat in their diet suggest that intestinal bacteria may play a role in pANCA status. If a larger sample size had been used in the present study, the potentially weak association between pANCA status and the feeding of a raw meat diet may have become a significant association.

A significant association between a positive result for pANCA and hypoalbuminemia was reported in another study.[4] Results of the present study did not reveal a significant association between these variables, which can be attributed to the use of healthy dogs. Serum albumin and total protein concentrations were high in the present study; 35 dogs had a serum albumin concentration that was above the reference range. This result may have been attributable to mild dehydration (because blood samples were collected in the morning) or to potential differences in the serum albumin and total protein concentration reference ranges in SCWTs, compared with the laboratory reference ranges.

The finding that SCWTs had significantly higher odds of having positive results for pANCA if they had a sibling with PLE or PLN suggests that pANCA may have potential use as an early marker of disease. However, the analysis that was performed in the present study did not reveal the mechanism of heritability of PLE or PLN. Interestingly, there was no influence of appearance of the Littman CMA in a pedigree on pANCA status in the present study. We have also found that the Littman CMA had no influence on PLE or PLN status in dogs with confirmed disease (unpublished data). Results of the present study revealed that the Littman CMA was present in almost all pedigrees of SCWTs in the United Kingdom; therefore, these pedigrees are extremely similar. The SCWT population in the United Kingdom is believed to be primarily descended from 4 CMAs, including the Littman CMA. Therefore, further analysis of the heritability pattern of PLE and PLN in SCWTs in the United Kingdom is warranted and should also include the other 3 CMAs.

Findings of a previous study[4] of SCWTs suggested that repeated sampling of dogs is necessary to achieve good predictive values of pANCA for PLE and PLN. Therefore, long-term monitoring of dogs that were tested for pANCA in the present study is needed before the risk that these dogs will develop immune-mediated diseases (including PLE and PLN) can be evaluated.

In the present study, dogs of breeds other than SCWT were not used as control animals, which is a limitation for extrapolation of the results reported here to other breeds of dogs. Dogs of multiple breeds should be included in future studies in which the use of pANCA as a diagnostic marker for immune-mediated or infectious diseases is investigated.

The potential for conditions such as PLE and PLN to have a familial pattern of inheritance in SCWTs[5] and other breeds[9–11] (Yorkshire Terriers, Norwegian Lundehunds, and Basenjis) suggests that an early marker of disease would be a useful clinical tool, even if that marker had limited specificity for prediction of disease. In dogs known to have a predisposition to PLE or PLN, a serologic marker of disease would allow early identification of dogs at high risk for developing disease. In such dogs, careful monitoring of health would allow early detection of PLE or PLN, and early initiation of palliative treatment for disease may increase life expectancy of the dogs and may improve their quality of life. If future studies reveal that pANCA testing is useful for prediction of immune-mediated diseases or for confirmation of infectious diseases, and the sensitivity and specificity of the test for prediction of disease are satisfactory, then development of a commercial ELISA for pANCA should be considered.

a. Win Episcope, version 2.0, European Commission's European Network on Veterinary Epidemiology and Economics (EPIDECON), Computer-aided Learning In Veterinary Education (CLIVE). Edinburgh, Scotland.
b. Breeders Assistant, version 4, Tenset Tech Ltd, Cambridge, England.
c. FITC, AbD Serotec AA132, Kidlington, Oxfordshire, England.
d. Stata, version 10, StataCorp LP, College Station, Tex.
e. SPSS, version 14, SPSS Inc, Chicago, Ill.

References

1. Peeters M, Joossens S, Vermeire S, et al. Diagnostic value of anti-*Saccharomyces cerevisiae* and antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease. *Am J Gastroenterol* 2001;96:730–734.
2. Wang G, Csernok E, de Groot K, et al. Comparison of eight commercial kits for quantitation of antineutrophil cytoplasmic antibodies (ANCA). *J Immunol Methods* 1997;208:203–211.
3. Allenspach K, Luckschander N, Styner M, et al. Evaluation of assays for perinuclear antineutrophilic cytoplasmic antibodies and antibodies to *Saccharomyces cerevisiae* in dogs with inflammatory bowel disease. *Am J Vet Res* 2004;65:1279–1283.
4. Allenspach K, Lomas B, Wieland B, et al. Evaluation of perinuclear anti-neutrophilic cytoplasmic autoantibodies as an early marker of protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers. *Am J Vet Res* 2008;69:1301–1304.
5. Littman MP, Dambach DM, Vaden SL, et al. Familial protein-losing enteropathy and protein-losing nephropathy in Soft Coated Wheaten Terriers: 222 cases (1983–1997). *J Vet Intern Med* 2000;14:68–80.
6. Melville-Walker SW, Smith KC, Elwood CM. Protein-losing enteropathy in a Soft-Coated Wheaten Terrier in the United Kingdom. *Vet Rec* 2004;154:440–441.
7. Vaden SL, Hammerberg B, Davenport DJ, et al. Food hypersensitivity reactions in Soft Coated Wheaten Terriers with protein-losing enteropathy or protein-losing nephropathy or both: gastroscopic food sensitivity testing, dietary provocation, and fecal immunoglobulin E. *J Vet Intern Med* 2000;14:60–67.

Unauthenticated | Downloaded 12/04/22 02:59 AM UTC

8. Vaden SL, Sellon RK, Melgarejo LT, et al. Evaluation of intestinal permeability and gluten sensitivity in Soft-Coated Wheaten Terriers with familial protein-losing enteropathy, protein-losing nephropathy, or both. *Am J Vet Res* 2000;61:518–524.
9. Peterson PB, Willard MD. Protein-losing enteropathies. *Vet Clin North Am Small Anim Pract* 2003;33:1061–1082.
10. Breitschwerdt EB. Immunoproliferative enteropathy of Basenjis. *Semin Vet Med Surg* 1992;7:153–161.
11. Landsverk T, Gamlem H. Intestinal lymphangiectasia in the Lundehund. Scanning electron microscopy of intestinal mucosa. *Acta Pathol Microbiol Immunol Scand A* 1984;92:353–362.

Correction: Daily endogenous cortisol production and hydrocortisone pharmacokinetics in adult horses and neonatal foals

In the report "Daily endogenous cortisol production and hydrocortisone pharmacokinetics in adult horses and neonatal foals" (*Am J Vet Res* 2012;73:68–75), there is an error on page 71 in the *P* value and the SD reported for foals in the last sentence of the first paragraph in the Results section.

The sentence should read as follows: The mean 24-hour cortisol concentration was significantly ($P = 0.04$) lower in foals (20 ± 1 ng/mL) than in adult horses (26 ± 6 ng/mL).

Unauthenticated | Downloaded 12/04/22 02:59 AM UTC