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Survey of the incidence of pectinate ligament dysplasia and glaucoma in the UK Leonberger population

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Abstract

Objective To determine the prevalence of pectinate ligament dysplasia (PLD) in UK Leonbergers and identify cases affected by glaucoma. Also, to define the spectrum of pectinate ligament (PL) appearance in this breed and determine whether gonioscopic monitoring should be recommended.

Animals studied Data were compiled from 78 prospective gonioscopy examinations performed by one author (GF) and retrospective analysis of 233 UK eye scheme certificates (2009–2014). Clinical cases of glaucoma in Leonbergers diagnosed by UK veterinary ophthalmologists, where gonioscopy of the fellow eyes or histology of affected eyes had been performed, were also reviewed.

Procedure In the prospective study, intraocular pressure was recorded prior to gonioscopy using a rebound tonometer. Gonioscopy was performed using a slit-lamp biomicroscope with a Koeppe goniolens. PLD was categorized according to the percentage of the iridocorneal drainage angle affected (grade 0 = <25% affected; grade 1 = 25–50% affected; grade 2 = 51–75% affected; and grade 3 = >75% affected), and the degree of narrowing of the angle was noted.

Results Of 78 dogs examined prospectively, 64/78 (82%) were grade 0, 7/78 (9%) were grade 1, 3/78 (4%) were grade 2, and 4/78 (5%) were grade 3. A large phenotypic variation was observed. Spearman's rank correlation showed a positive correlation between age and severity of PLD ($P < 0.0055$). 52 (22%) of Leonbergers examined under the UK eye scheme 2009–2014 were affected by PLD. Five clinical cases of glaucoma were reviewed where gonioscopy had been performed and one where histology was performed. All individuals had grade 3 PLD with gonioscopy of the contralateral eye or severe goniodysgenesis with histological sections of the affected eye.

Conclusion This survey suggests the prevalence of PLD is sufficient to justify ongoing screening of Leonbergers.

Key Words: goniodysgenesis, gonioscopy, iridocorneal angle, Leonberger, pectinate ligament dysplasia, primary glaucoma

INTRODUCTION

Glaucoma refers to a group of potentially painful and blinding diseases resulting in progressive death of retinal ganglion cells and their axons.[1] Categorization into 'primary' (i.e., no antecedent or coincident ocular disease) or 'secondary' glaucomas, with further subcategorization according to the state of the iridocorneal angle (open, narrow, closed) and the antecedent cause, remains the most commonly utilized classification system in veterinary practice. Unlike our human counterparts, the uniting feature of glaucoma in dogs is an elevation of intraocular pressure (IOP) above 'normal'.[1,2] This increase in intraocular pressure typically occurs due to increased resistance to outflow of aqueous humor altering the balance with rate of production by the ciliary body.[3]

The pectinate ligament (PL) is the anterior-most structure of the iridocorneal drainage angle (ICA), where 85% of aqueous humor drainage occurs in the dog via the conventional aqueous outflow pathway.[4] Pectinate ligament dysplasia (PLD, alternatively referred to as goniodysgenesis) refers to consolidation of the normally

fine pectinate ligament strands into short, broad bands or sheets of tissue, with or without flow holes. PLD has been related to the development of primary narrow and closed-angle glaucomas in a number of breeds, including the Basset Hound,[5] Bouvier des Flandres,[6] Japanese Shiba Inu,[7] Flat-Coated Retriever,[8,9] American Cocker and Cocker Spaniel,[10] English and Welsh Springer Spaniel,[11,12] and Samoyed [13] but not to our knowledge in the Leonberger. Concurrent narrowing of the ICA has also been reported in affected breeds.[12,13]

The meridionally oriented, branching comb-like strands of the PL span the opening to the ciliary cleft, arising from the iris base and inserting into the posterior aspect of the peripheral cornea. A primary row of strands, most prominently viewed on gonioscopy, has been identified, with an interconnected accessory row posterior to this more readily viewed by scanning electron microscopy.[14] In the healthy eye, aqueous humor flows from the posterior chamber, through the pupil into the anterior chamber circulating toward the ICA. Beyond the PL, aqueous humor flows into the uveal trabecular meshwork and then into the corneoscleral trabecular meshwork eventually reaching the aqueous plexus veins, scleral venous plexus, and episcleral veins leaving the eye.[3,9]

The use of gonioscopy to evaluate the ICA in veterinary patients was first described in the 1960s.[15,16] Since then, gonioscopy has become an important part of the clinical assessment of glaucomas and screening of breeding stock. Although not all individuals possessing narrowed iridocorneal angles go on to develop clinical glaucoma, routine examination of young animals and selective breeding have been encouraged in an attempt to eradicate the disease.[10] Such monitoring may reveal more about the development and progression of this disease. It remains likely, however, that the gonioscopically visible anomalies may combine with other changes in the deeper regions of the angle responsible for the glaucoma.[17]

Pre- and postnatal PL development and remodeling have been reported in electron microscopic and histological studies which describe the process of rarefaction of sheets of mesenchymal tissue and formation of fine filaments, and postnatal ICA development was considered to be complete at postnatal day 21.[18,19] However, a recent longitudinal study demonstrating progression of PLD in the Flat-Coated Retriever suggests that significant postnatal PL remodeling continues throughout adulthood, indicating that serial gonioscopy in breeding stock is warranted.[20] Progression of PLD throughout adulthood may also suggest reclassification with a new term other than dysplasia.[20] Whether this change constitutes descemetization of the PL as suggested by a retrospective histopathological survey conducted by Smith *et al.* has not been investigated.[2] It is also possible that changes in the PL are merely a marker of more significant change in the ciliary cleft given the finding of fine pores within mesenchymal sheets using scanning electron microscopy (SEM).[14]

The Leonberger in the United Kingdom is a numerically small breed, with 391 UK Kennel Club registrations in 2013 and 3720 registered over the last 10 years (http://www.thekennelclub.org.uk/registration/breed-registration-statistics/). In 2012, a small number of clinical cases of primary narrow angle glaucoma associated with PLD in Leonbergers were reported to the British Veterinary Association/Kennel Club/International Sheep Dog Society (BVA/KC/ISDS) eye scheme (Equivalent to CERF/ECVO certification). A near 50% prevalence of pectinate ligament dysplasia (PLD) in this breed was suggested by a brief review of the BVA/KC/ISDS eye scheme data (BVA, Mason, I unpublished data) from 2009 to 2011.

The main purpose of this study was to conduct a prospective gonioscopic survey to determine the prevalence and phenotypic spectrum of PLD in the UK population of Leonbergers. In addition, we performed a retrospective analysis of BVA/KC/ISDS eye scheme data to more accurately determine the incidence of PLD previously reported in this breed. Finally, we aimed to document clinical cases of glaucoma seen in this breed and to determine whether an ongoing program of gonioscopic monitoring should be recommended.

MATERIALS AND METHODS

In the prospective survey, iridocorneal angles of 78 Leonbergers were examined by one author (GF) at breed club events in varying locations between August 2012 and July 2014. These events were advertised and organized by the Leonberger Breed Club of Great Britain and owners gave informed consent for the testing as part of the survey. Dogs with a history of antecedent intraocular disease were excluded, as were any dogs that would not tolerate gonioscopic examination. Prior to gonioscopy, ophthalmic examination including slit-lamp biomicroscopy and indirect ophthalmoscopy was performed, and intraocular pressure was recorded using a rebound tonometer (TonoVet, Icare, Helsinki, Finland). Gonioscopy was performed in conscious, unsedated dogs using a slit-lamp biomicroscope (Kowa SL-14, Kowa Optimed Europe Ltd, Berkshire, UK, or Keeler portable slit-lamp, Animalcare Ltd, York, UK) set with a diffuse, 1/16 or moderate level illumination at ×10 magnification. A 17 or 19 mm Koeppe goniolens (Ocular Instruments inc., Bellevue, WA, USA) was placed following topical anesthesia with proxymetacaine 1% (Bausch & Lomb, Chauvin Pharmaceuticals Ltd, Aubenas, France). Where possible, the eyelid margin was held in the groove of the lens, but in many individuals, the relatively loose eyelid typical of the breed required gentle manual stabilization of the lens. Given the large size of the eye in this breed, the majority of cases were examined using the 19-mm goniolens. The opening of the ICA was assessed through 360° using a quadrant based system starting ventrally and progressing in a clockwise fashion (illustrated in Fig. 1). PLD was categorized

according to the percentage of the iridocorneal angle affected (grade 0:<25% affected; grade 1: 25–50% affected; grade 2: 51–75% affected; and grade 3:>75% affected). This relatively broad grading system was intended to reduce subjectivity of grading by avoiding the estimation of narrower percentage ranges. Such a system allows for small areas of pectinate ligament consolidation (e.g., that associated with the medial entry of the posterior ciliary artery) that are considered within normal limits. Goniophotograph examples of each grade are provided in Fig. 2. The degree of narrowing of the angle was noted as either 'closed,' 'narrow,' or 'open.' Both eyes were examined, and in those cases in which the grade varied between eyes, the higher grade was consistently used for the data analysis. Cheek swabs for DNA were collected from a range of 'normal' (grade 0), 'moderately affected' (grades 1&2), and 'severely affected' (grade 3) cases. These samples are being processed by the genetics laboratory at the Animal Health Trust in preparation for performing a genomewide association study (GWAS).

A Spearman's rank correlation was performed to test the null hypothesis that there was no difference between grade of PLD and age, with $P < 0.05$ as significant.

The retrospective survey involved analysis of 233 UK eye scheme certificates from 2009 to 2014. Certificates were reviewed and the findings recorded as either 'affected' or 'unaffected' according to the BVA panel guidelines.[21] As no percentage of affectation is stated under the BVA scheme, the data for the retrospective survey were nonquantifiable.

A third subpopulation of six Leonbergers diagnosed with primary closed-angle glaucoma was not included in the main survey population to avoid selection bias. Case details were provided by members of the British Association of Veterinary Ophthalmologists (BrAVO), following a short presentation during a meeting and an email request for information. Gonioscopy and histology results

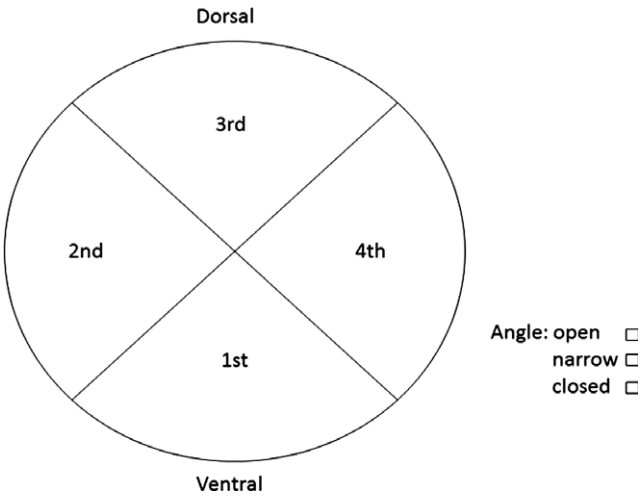

Figure 1. Schematic diagram of system of gonioscopic examination system employed in the prospective survey. Examination commenced in the ventral quadrant, moving either medially or laterally (depending on left or right eye), then dorsally. A note was made of the overall width of the angle as open, narrow, or closed.

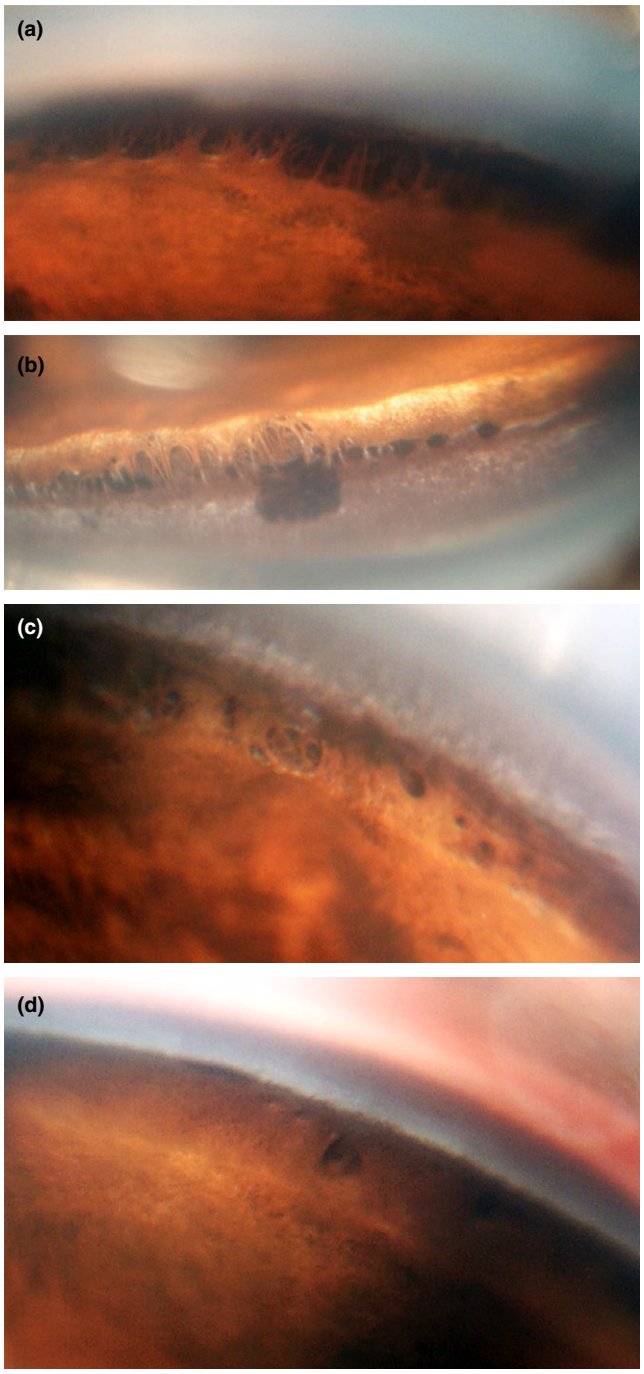

Figure 2. The following series of images are goniophotographs of examples from each grade within the prospective study. The images were obtained in conscious dogs, and so the angle of imaging was as consistent as possible but could not be readily standardized. These images only demonstrate a small area of the angle and are representative rather than illustrative. (a) Grade 0 (open angle, <25%). (b) Grade 1 (open angle, 25–50% affected). (c) Grade 2 (open angle, 51–75% affected). (d) Grade 3 (open angle with extensive sheeting and few flow holes >75% affected).

available for these dogs were reported to the authors and cheek swabs for DNA collected where possible. Three other dogs with glaucoma but no gonioscopic assessment of the fellow eyes or histology on affected eyes were ruled out of consideration.

RESULTS

The results of gonioscopic examination for 78 individuals (44 females and 34 males) were graded. The mean age was 4 years (range 0.4–11 years), 32 dogs were <2.5 years, 16 were 2.5–5 years, 18 were 5–7.5 years, eight were 7.5–10 years, and two were >10 years of age. Owners failed to provide a date of birth or registration details for two dogs. Both eyes were examined in all dogs; in four cases, the grading differed between eyes in the same dog.

Table 1 shows the distribution of PLD grading for the prospective survey. A total of 64/78 (82%) were grade 0, 7/78 (9%) were grade 1, 3/78 (4%) were grade 2, and 4/78 (5%) were grade 3.

The angle was noted as narrowed or closed in 15/78 (19%) cases in the prospective study. The distribution of narrowing between grades and the severity is reported in Table 1.

Intraocular pressure was measured in 72 individuals (144 eyes) in the prospective study (Table 1). There was no increase in intraocular pressure associated with increased severity of PLD at the time of examination.

Spearman's rank correlation was performed for the relationship between age and PLD score (Fig. 3). Due to missing dates of birth for two individuals, a sample of 76 Leonbergers were included in this analysis. There was a positive correlation between age and severity of PLD, which was statistically significant ($r_s = 0.3158$, $P < 0.0055$) (Fig. 3).

Results of 233 examinations (141 females and 92 males) performed under the BVA/KC/ISDS eye scheme 2009–2014 were reviewed (Table 2). The mean age was 3 years (range 0.27–10.49 years), 119 dogs were <2.5 years, 71 dogs were 2.5–5 years, 38 were 5–7.5 years, four were 7.5–10 years, and one was >10 years of age. A total of 52 (22%) of Leonbergers examined were affected by PLD; of these, three individuals were affected in one eye, but unaffected in the other. A comment regarding narrowing of the angle was made in 44/233 (19%) cases in the retrospective survey. In 20 of the affected dogs, the examiner had commented that they were severely affected by PLD in at least one eye. A total of 181/233 (78%) Leonbergers were unaffected by PLD according to the BVA/KC/ISDS eye scheme in the same time period.

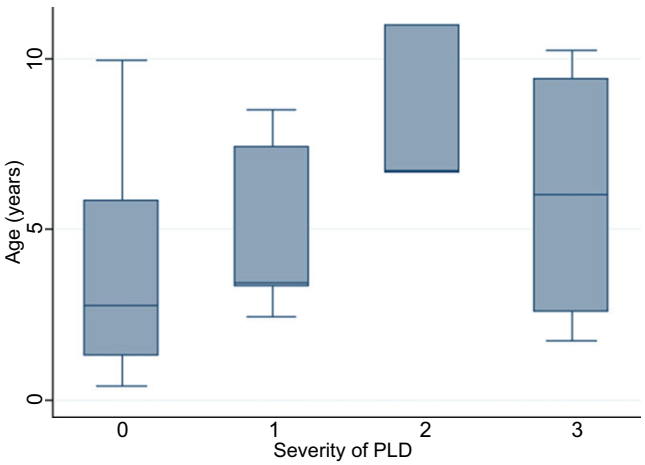

Figure 3. Box and whisker plots to illustrate Spearman's rank correlation for the relationship between age and PLD score for the prospective study. Due to missing date of birth for two individuals, a sample of 76 Leonbergers were included in this analysis. There was a positive correlation between age and severity of PLD, which was statistically significant ($r_s = 0.3158$, $P < 0.0055$).

The distribution of narrowing between affected and unaffected dogs is reported in Table 2.

Of six individuals reported to the authors to be affected by glaucoma, five underwent gonioscopic examination, (two by one author (GF) and the other three by BVA panelists) and all of these cases were classified as grade 3 in the contralateral eye (the affected eye being unexaminable by gonioscopy due to diffuse corneal edema). A histopathology report suggesting PLD as the cause of glaucoma was available for one other case. Gonioscopy had not been possible in this case due to the presence of bilateral corneal edema. The age at onset of glaucoma

Table 1. Survey results for the prospective population examined by one author (GF). In this population, the angle was graded 0–3 for severity of PLD according to the system illustrated in Figure 1. Where possible, intraocular pressure was measured before gonioscopy. Narrowing was recorded as open, narrow, or closed. The total number of narrow or closed angles in each group is recorded in this table; both cases with closed angles had normal intraocular pressures

Angle Grade	Frequency (*n*)	Percentage of study population (%)	Mean age of dogs in years (range)	Ratio of males to females	Mean IOP mmHg (no. of eyes)	Angle narrowing (no. of eyes)	
						Narrow	Closed
0	64	82	3.60 (0.38–9.53)	1:1.6	14.2 (112)	7	0
1	7	9	4.88 (2.42–8.51)	1:0.75	14.7 (18)	4	0
2	3	4	8.12 (6.64–11.01)	No females	13.2 (6)	1	1
3	4	5	6.01 (1.73–10.25)	1:1	13.1 (8)	1	1
Total	78	100					

Table 2. Survey results for the retrospective portion of the study compiled from BVA/KC/ISDS eye test results. As the scheme does not have a grading scale, results could only be reported as affected or unaffected

Angle PLD status	Frequency (*n*)	Percentage of study population (%)	Mean age of dogs in years (range)	Ratio of males to females	Angle narrowing (no. of eyes)	
					Narrow	Closed
Unaffected	181	78	2.83 (0.27–10.49)	1:1.48	16	0
Affected	52	22	3.75 (0.49–6.89)	1:1.89	21	7
Total	233	100				

ranged from 3.86 to 8.50 years, with a mean age of 6.09 years. The glaucoma group consisted of five females and one male (1:5 male:female ratio).

DISCUSSION

In our prospective study, 14/78 (18%) of Leonbergers were affected by PLD to some degree (grades 1–3). Of these, 4/78 (5%) were severely affected (grade 3). A great amount of phenotypic variation was noted during the prospective survey in this breed, ranging from very wide angles containing fine, pale strands of pectinate ligament, and a greater number of narrower angles, with short, thick, and darkly pigmented strands of PL. A survey of 500 dogs from a mixture of breeds and cross-breds by Bedford in 1977 reported similar variations both between breeds and individuals within breeds.[22]

In our retrospective survey, 52/233 (22%) of cases were classed as affected by PLD. This represents a slightly higher prevalence than seen in the prospective study and may reflect a slight selection bias, with individuals more likely to be presented for testing whether a relative is known to be affected.

In our clinical glaucoma study, all glaucomatous dogs examined were classed as grade 3 in the contralateral eye. This correlates with previous studies linking glaucoma to PLD.[7–9] The contralateral eyes were all normotensive at presentation, with the time to developing bilateral disease ranging from 10 days to 1 year. Due to the small population affected, the link between glaucoma and PLD could not be examined for statistical significance in this breed.

In the prospective survey, on each testing day, there were individuals that could not be examined as they would not tolerate placement of a goniolens. There were a large number of young dogs within this survey due to breeder encouragement for owners of recent litters to attend. This skewed age population may have altered the distribution of results seen in this survey; however, the results of the Spearman's rank correlation suggest a significant association between increasing age and severity of PLD. With the nonlinear nature of the data in this study, it is not possible to determine whether this represents progressive change as observed in the Flat-Coated Retriever.[20] Changes in the pectinate ligament with increasing age have been demonstrated in the English Springer Spaniel, but not the Samoyed.[12,13] Although a positive relationship between PLD and glaucoma has been shown, understanding of this relationship remains poor.[12] In the present study, all reported cases of glaucoma had severe PLD in the contralateral eye. The age range of 3.86–8.50 years might suggest, however, that clinically age is not the only risk factor leading to development of glaucoma.

The angle was noted as narrow or closed in 15/78 (19%) cases in the prospective study and 44/233 (19%) cases in the retrospective survey, similar to the proportion observed by Bjerkas *et al.* in the English Springer Spaniel (ESS) in which 17.9% of dogs showed narrowing, and in which PLD was found to have a statistically significant effect on the width of the ICA.[12] It is interesting to note that a larger percentage of individuals with angle narrowing in the present study were unaffected by PLD, 7/15 (47%) in the prospective and 16/44 (36%) in the retrospective Leonberger surveys compared with just 4.3% in the English Springer Spaniel. Although a relationship between PLD and angle narrowing has been demonstrated, it remains unclear whether these two factors are contributing directly to the development of primary glaucoma or serving as markers for disease at another level of the aqueous humor outflow pathways.[12]

More females than males were affected by both PLD and glaucoma in this survey, and this concurs with findings in other breeds and also with human primary glaucoma cases.[23]

Three cases in the retrospective survey and four cases in the prospective study were found to have one eye less affected by PLD than the other. In his study of the development of the PL of the dog by scanning electron microscopy, Martin noted that variation in development between eyes existed between littermates and even within the same eye.[18] These differences highlight the importance of examining the entire drainage angle of both eyes during gonioscopic examination.

With the large eye in the Leonberger, the author found when performing gonioscopy, movement of the lens toward the quadrant under examination was necessary to visualize the angle. Changing the angle of observation in this way was described by Martin in 1969.[15] Caution must be taken, however, not to apply sufficient pressure to force the angle open by increasing aqueous outflow.

The various grading systems for gonioscopic studies only emphasize the subjectivity of this method of assessment and lack of 'perfect' system for quantifying change. Even with goniophotography, the position of the goniolens relative to the ICA and angle of image capture will influence perceived width of the drainage angle introducing unavoidable variability to findings. During this survey, only selected individuals tolerated goniophotography conscious and upright, and it would not have been possible to photograph every case without sedation. Despite the development of new technologies such as high-frequency ultrasonography and anterior segment optical coherence tomography, there is currently no clinical technique that allows objective examination of the entire ICA structure.[24,25] As technology develops, however, we hope to gain more detailed knowledge of the changes beyond the pectinate ligament occurring before and during narrowing of the ICA. The difficulty with these techniques is achieving repeatable representative images in the conscious animal.

There are several limitations in this study. Although the IOP in all cases tested during the survey was normal, it was not possible to standardize these measurements. Dogs presented throughout testing days and so readings were performed at varying times of day. Seventy-two readings were performed by one examiner (GF) with the same instrument but with owner restraint, although guided by the tester, not being uniform. At the time of the initial analysis of BVA eye testing data, gonioscopy was only performed if requested by owners. It is possible that this created a selection bias with dogs presented likely to be related to those known to be affected. In 2012/2013, there was a surge in the number of Leonbergers presenting for gonioscopy following publicity regarding the occurrence of glaucoma in the breed, creating the shift in prevalence seen during this time. Analysis of BVA eye test forms was only possible in cases where a clear description of the angle was provided. It is not a requirement of this scheme to describe the angle beyond 'affected' or 'unaffected' and grading by panelists is currently discouraged. Under the BVA scheme, eyes with mild PLD alone or mild angle narrowing alone are classed as 'unaffected,' whereas a combination of both PLD and angle narrowing are classed as 'affected,' as are marked PLD and marked angle narrowing alone. There are currently 40 members of the panel, and so inevitably, there is likely to be variation in gonioscopy technique. However, all panelists are experienced ophthalmologists and have clear guidelines for examination.[21] It is likely that cases of glaucoma in this breed are underrepresented, with cases unreported when treated in general practice and enucleated without ocular histopathology.

In conclusion, the Leonberger in the United Kingdom shows a high prevalence of pectinate ligament dysplasia, with a number of primary glaucoma cases presenting clinically associated with PLD. These findings warrant inspection of the pectinate ligament by gonioscopy at prebreeding eye testing.

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