

# The effect of repeated eye examinations and breeding advice on the prevalence and incidence of cataracts and progressive retinal atrophy in German dachshunds over a 13-year period

Sarah Koll,<sup>\*</sup>† Sven Reese,<sup>‡</sup> Ivica Medugorac,<sup>§</sup> Carsten U. Rosenhagen,<sup>¶</sup> Rick F. Sanchez<sup>†</sup> and Roberto Köstlin<sup>\*</sup>

<sup>\*</sup>Clinic for Small Animal Surgery and Reproduction, Veterinary Faculty, Ludwig Maximilians University Munich, Veterinärstr. 13, Munich, Germany; <sup>†</sup>Royal Veterinary College, University of London, Hawkshead Lane, North Mymms, Hertfordshire AL9 7TA, UK; <sup>‡</sup>Institute of Veterinary Anatomy, Histology and Embryology, Veterinary Faculty, Ludwig Maximilians University Munich, Veterinärstr. 13, Munich, Germany; <sup>§</sup>Chair of Animal Genetics and Husbandry, Veterinary Faculty, Ludwig Maximilians University Munich, Veterinärstr. 13, Munich, Germany; and <sup>¶</sup>Dortmunder Kreis – Association for Diagnosis of Inherited Eye Diseases in Animals (DOK), Hoher Wall 20, 44137 Dortmund, Germany

Address communications to:

S. Koll

Tel.: 44 (0)1707 666365

Fax: +44 (0)1707 649384

e-mail: skoll@rvc.ac.uk

## Abstract

**Objective** To analyze the change in prevalence and incidence of hereditary eye diseases (HED) in dachshunds due to breeding regulations based on biennial examinations performed by the German panel of veterinary ophthalmologists (DOK) from 1998 to 2011.

**Animals included** A total of 12 242 dachshunds examined by the DOK and pedigree data of 318 852 dachshunds provided by the German Dachshund Club (DTK).

**Procedures** The prevalence of congenital cataract (CC), distichiasis (DIST), hereditary cataract (HC), persistent pupillary membranes (PPMs), persistent hyperplastic tunica vasculosa lentis / persistent hyperplastic primary vitreous (PHTVL/PHPV), progressive retinal atrophy (PRA), retinal dysplasia (RD), and findings such as fiberglass-like cataract (FGC) and prominent suture lines (PSLs) was analyzed. The significance (P), confidence interval (CI), odds ratio (OR), relative risk (RR) and inbreeding coefficients (F) were calculated and  $P < 0.05$  was considered significant. The incidence was evaluated based on affected dogs within birth cohorts from 1993 to 2006.

**Results** The prevalent conditions studied were as follows: CC 0.5%, DIST 6.7%, HC 3.9%, PPMs 8.4%, PHTVL/PHPV 0.4%, PRA 1.5%, RD 0.2%, FGC 2.2%, and PSL 1.5%. The incidence of PRA decreased significantly from 6.0% to 0.6% for dogs born from 1993 to 2006, while HC showed a decreasing trend from 8.7% to 3.1%. More males than females were diagnosed with HC and PRA. Dachshunds with HEDs had an F that was not significantly higher than that of healthy dachshunds.

**Conclusions** The decreasing incidence of PRA and HC in dachshunds supports the use of frequent HED examinations in combination with breeding control.

**Key Words:** cataract, dachshund, Hereditary eye disease, inbreeding coefficient progressive retinal atrophy

## INTRODUCTION

Hereditary eye diseases are a concern as they may affect the breeding fitness of many dog breeds. Veterinary ophthalmologists and breeding clubs share an interest in standardized ophthalmic examinations as a tool to evaluate prevalence and incidence of hereditary eye diseases. The European College of Veterinary Ophthalmologists (ECVO) runs a program for the diagnosis and control of

presumed inherited eye diseases (PIED). It is called the hereditary eye disease scheme, and it is referred to as ‘the Scheme’ or the ‘ECVO Scheme’. It provides definitions, guidelines, advice, and information concerning PIED, as described in the ECVO Manual for PIED of dogs and cats. European Specialists in Veterinary Ophthalmology (ECVO Diplomates) and specifically trained veterinarians (European Eye Scheme Examiners or ESE) perform the eye examinations in the ECVO Scheme.<sup>1</sup> The German

Eye Panel, known as the 'Dortmunder Kreis' (DOK), has been part of the ECVO Scheme since 1998 and provides dog owners with eye examinations for hereditary eye diseases (HED) and PIED, using the standardized examination form of the ECVO. The DOK consists of approximately 80 veterinarians working in private practice and universities.<sup>2</sup> To become a DOK member, a standardized admission procedure has to be passed successfully, including giving evidence of advanced training in ophthalmology with ophthalmic instrumentation and passing a theoretical and practical examination.

Historically, since 1995, dogs bred by the 'German Dachshund Club 1888 e.V.' (DTK) underwent compulsory HED examinations every 2 years. The examinations were carried out by either a veterinary surgeon with an interest and a qualification in ophthalmology or a member of the DOK. However, from 2003 onward, the DTK only accepted examinations carried out by members of the DOK.<sup>3,4</sup> The HED/PIED examinations by members of the DOK in Germany provided data of 12 242 individual dachshunds, examined between 1998 and 2011. Only dogs that were diagnosed as affected by HC or PRA and not their offspring were excluded from breeding.<sup>5</sup> A cataract was defined as any opacity of the lens and/or its capsule and was assumed to be hereditary unless it was associated with trauma, inflammation, specific metabolic diseases or nutritional deficiencies.<sup>6</sup>

Gresky and Mueller studied the prevalence of PRA and HC in dachshunds from Germany in 2004 and 2007. Gresky demonstrated the prevalence of 4.6% for HC and 1.6% for PRA in dachshunds and Mueller found a prevalence of 3.38% for HC in wild-boar-colored WHDs.<sup>7,8</sup> The Genetics Committee of the American College of Veterinary Ophthalmologists (ACVO) categorized cataracts and PRA as PIED in dachshunds in 2013 and published the prevalence in different dachshund varieties in 2014, based on data collected by ACVO Diplomates over 2 decades.<sup>9,10</sup> Although further studies in dachshunds from Germany were not conducted until now and Gresky and Mueller did not report the incidence of these diseases in their studies, the DTK felt there was evidence the prevalence of HC and PRA were lower when subjectively compared to earlier years, at the start of compulsory eye examinations in Germany. As a result, the DTK decided to abandon compulsory biennial examinations in 2013 in favor of voluntary examinations, except for one compulsory examination that was carried out before the breeding license was issued.<sup>5</sup>

The purpose of this study was to report the prevalence and the incidence of a selection of hereditary eye diseases in nine dachshund varieties through a detailed retrospective analysis of the data collected by members of the DOK between 1998 and 2011, with a focus on PRA and HC. Furthermore, the study aims to compare inbreeding coefficients ( $F$ ) between healthy and affected dachshunds registered in the DTK.

## MATERIALS AND METHODS

### *Database of the DOK*

The data of 16 447 HED/PIED examinations that were performed exclusively by certified members of the DOK/ECVO were included in this study. The examination methods for the ECVO Scheme, which includes examinations by members of the DOK, were carried out according to the ECVO protocol, which is found in the ECVO website<sup>11</sup> and according to the procedure notes of the ECVO HED committee.<sup>12</sup> The following has been paraphrased from the ECVO website: All animals presented under the Scheme have a general examination of the eye and adnexa in darkened surroundings and a short-acting mydriatic is used. For those breeds specifically examined for pectinate ligament abnormality, persistent pupillary membrane, or iris hypoplasia, examination of the anterior segment must be conducted before the use of a mydriatic. Slit-lamp biomicroscopy (with at least 10 × magnification) and indirect binocular ophthalmoscopy are minimal requirements of the ophthalmic exam. The use of other equipment is optional. A list of breeds and their conditions is published in the ECVO Manual: Guidelines, and revised regularly by the ECVO Hereditary Eye Diseases committee.

The original method of identification of each animal was the animal's microchip or tattoo. The ID presented by the owners at the time of the DOK examination had been issued by the DTK and was verified against the data found in the studbook, including breeding name, signalement, microchip number, and date of birth. As some dogs had multiple eye examinations throughout their lives, these 16 447 examinations were carried out on a total of 12 242 dachshunds. The analysis included the following parameters: breeding name and breed registry ID of the dog, size variant and hair type, date of birth, sex, total number of examinations and their result, and the date of the last examination. The result of the most recent examination was used for the statistical evaluation. The data of 11 431 dachshunds were verified against the data that each dog had in the DTK studbook. Unfortunately, dogs that originated from other breeding clubs ( $n = 811$ ) had their information in other studbooks that were not available for verification.

The relevant size varieties from smallest to largest were rabbit, miniature, and standard dachshunds. The relevant hair types were wire haired (WHD), long haired (LHD), and smooth haired (SHD). The combination of size and hair type represented the nine varieties of dachshunds as defined by the Fédération Cynologique Internationale (FCI).<sup>13</sup>

The diseases reviewed in this study included congenital cataract (CC), distichiasis / ectopic cilia (DIST), noncongenital hereditary cataract (HC), persistent pupillary membranes (PPMs), persistent hyperplastic tunica vasculosa lentis / persistent hyperplastic primary vitreous (PHTVL/PHPV),

progressive retinal atrophy (PRA), and retinal dysplasia (RD). Hereditary eye diseases were diagnosed by clinical history and ophthalmic examination, as described previously. Additional examinations, for example ERG, were optional and not mentioned in the available data. Descriptors for cataracts were adopted from the ECVO examination certificate (cortical, posterior polar, anterior suture line, punctata, and nucleus).<sup>14</sup> Opacities of the lens such as fiberglass/crystal-like cataracts (FGC) (crystal-like changes distinctly in the nucleus not to be confused with pulverulent-like cataracts) and prominent suture lines (PSLs) were evaluated out of interest and were not counted as HED/PIED, according to the ECVO HED procedure notes.<sup>12</sup> Data regarding FGCs and PSLs were taken from the descriptive comments made by the examiners on the ECVO examination certificate and noted as either present or absent. No further information regarding these findings (e.g., age at diagnosis, localization) was given. In case of doubt of a PIED, a grading of 'suspicious' was awarded. As further changes could confirm a diagnosis, re-examination would be advised in at least 6 months, but up to 12 months. Some animals would be re-examined at a panel meeting.<sup>12</sup>

The data were translated into numeral codes for statistical evaluation. After a control for plausibility, IBM SPSS Statistics for Windows (version 21.0; IBM Corp., Armonk, NY, USA) and  $\chi^2$  test by ACOMED (version 3b; Leipzig, Germany, 2008) were used for the statistical analysis. The level of significance ( $P$ ) was 0.05 where lower values were considered significant. Confidence intervals (CI) were stated at a 95% confidence level. The Holms–Bonferroni method was used when the significance between multiple groups was compared.<sup>15</sup> The BIAS EPSILON program (version 10/04-07/2013, Goethe U Frankfurt, Germany) was used for the calculation of relative risk (RR) and odds ratio (OR).

Dogs with the same year of birth were referred to as a 'birth cohort'. The examination results of affected dogs of every birth cohort from 1993 to 2006 were reviewed to demonstrate the incidence of HC and PRA.

The definition of incidence is the number of new cases of a disease occurring during a period of time in a population at risk.<sup>16</sup> The earlier cohorts (1985–1992) provided a low number of examinations (<100 per year) and dogs born from 2007 to 2011 were not included as they were presumably too young to be affected by the diseases studied, and their inclusion could have led to falsely lowered results. Only new cases of the diseases studied were counted with each birth cohort from 1993 to 2006.

#### *Database of the DTK*

There were a total of 298 149 dachshunds included. The DTK provided pedigrees of the 318 852 dachshunds listed in the studbook from 1888 to 2011. This database was used for identification and verification of the dachshunds from the DOK database and for the calculation of  $F$ . The

data used for each dog included the breed registry ID, date of birth, sex, and the ID of its mother and father and were controlled for plausibility. A total of 20 703 dogs had to be excluded from the study, as their data were incomplete. Due to the unexpected effects of a software update, many dogs of the older generations showed as having unknown birth dates. To avoid information gaps, the DTK gave all of these dogs the same birth date (01.01.2002). This created a challenge for the restoration of the pedigree constellations for the calculation of  $F$ s. To solve this, the breed registry ID of individual dogs and their parents was used to help list dogs in separate generations that were designated as parent generations. This provided pedigree information up to the 22nd generation. Complete pedigree information up to the 5th generation was given for most, but not all of the dogs.

#### *Inbreeding coefficients ( $F$ )*

The measure  $F$  is the coefficient of inbreeding, which is the probability that the two genes at any locus in an individual are identical by descent. Animals whose parents were unrelated show the  $F$ -value 0 or close to 0. Animals inbred to one common grandparent show  $F = 0.125$ , which means 12.5% of the genes coming to this individual by the maternal or paternal gamete are identical by descent or are copy of the same grandparental chromosome. In theory, the maximal  $F$ -value is 1.0, that is, the animal is homozygous for 100% of loci and all maternal and paternal chromosomes come from the same origin.<sup>17</sup>

Data of the DTK were aligned with data of the DOK by assigning each dog with its individual ID. Four groups were created. Group 1 consisted of dachshunds with PRA ( $n = 111$ ). Group 2 consisted of dachshunds with HC ( $n = 351$ ). Group 3 ( $n = 165$ ) and 4 ( $n = 492$ ) were comparison groups consisting of half-brothers and half-sisters of those with PRA and HC, respectively. For all groups, average inbreeding coefficients were calculated according to Henderson and later on compared.<sup>18</sup> An additional calculation was performed later, after reducing Group 2 to all dogs with a complete pedigree till the fifth generation ( $n = 315$ ). The formula  $F_X = \sum (1/2)^n (1 + F_A)$  was used to calculate the  $F$ .<sup>17</sup> The ' $n$ ' in the formula is the number of ancestors in an inbreeding loop, and ' $F_A$ ' refers to the coefficient of the ancestors. The comparison of the groups was carried out by  $t$ -tests using SAS procedure  $t$ -test (version 9.3; SAS Institute Inc. Cary, NC, USA).

## RESULTS

A total of 12 242 dachshunds were examined, 8268 females (68%) and 3974 males (32%). Table 1 displays the prevalence of HED/PIEDs in all dachshunds, not separated by variety or hair type. Table 2 shows the absolute and percentile prevalence of HC and PRA in dachshunds, examined by the DOK in the years 1998–2011.

**Table 1.** Absolute and percent prevalence of HEDs in dachshunds, examined by the DOK in Germany in the years 1998–2011

HED	Free	Affected	Percent and CI 95%
Congenital cataract	12175	65	0.5 (0.4–0.6)
Distichiasis/ectopic cilia	11414	825	6.7 (6.3–7.1)
Hereditary cataract	11758	481	3.9 (3.5–4.2)
Persistent pupillary membranes	11172	1029	8.4 (7.9–8.9)
PHTVL/PHPV	12162	45	0.4 (0.2–0.4)
Progressive retinal atrophy	12053	187	1.5 (1.3–1.7)
Retinal dysplasia	12188	30	0.2 (0.1–0.3)
Others			
Fiberglass-like cataract	11962	272	2.2 (1.9–2.4)
Prominent suture lines	12049	186	1.5 (1.3–1.7)

**Table 2.** Absolute and percent prevalence of HC and PRA in the various Dachshund varieties, examined by the DOK in Germany in the years 1998–2011

Dachshund varieties	<i>n</i> -total	Progressive retinal atrophy		Hereditary cataract		
		<i>n</i> -affected	%	<i>n</i> -affected	%	
Rabbit	Smooth haired	74	0	0	1	1.4
	Long haired	133	3	2.3	7	5.3
	Wire haired	282	3	1.1	7	2.5
	Total	489	6	1.2	15	3.1
Miniature	Smooth haired	211	2	0.9	3	1.4
	Long haired	636	14	2.2	26	4.1
	Wire haired	1284	6	0.5	38	3.0
	Total	2131	22	1.0	67	3.1
Standard	Smooth haired	1247	11	0.9	28	2.2
	Long haired	1403	32	2.3	69	4.9
	Wire haired	6970	116	1.7	302	4.3
	Total	9620	159	1.7	399	4.1
Total	Smooth haired	1532	13	0.8	32	2.1
	Long haired	2172	49	2.3	102	4.7
	Wire haired	8536	125	1.5	347	4.1
	Total	12240	187	1.5	481	3.9

Because HC and PRA were the focus of this study, the data for these conditions were analyzed with the following results:

A total of 3.9% (CI<sub>95</sub>: 3.5–4.2%) of dachshunds in this study were affected by HC, which represented a total of 191 (4.8%) of all the examined males and 289 (3.5%) of all the examined females. Males had a significantly higher risk of being affected (RR: 1.37, OR: 1.39,  $P < 0.001$ ). The prevalence of HC was 3.1% in miniature and rabbit dachshunds and 4.1% in standard dachshunds, but this difference was not significant (miniature vs. rabbit dachshunds  $P = 0.935$ , standard vs. rabbit dachshunds  $P = 0.485$ , miniature vs. standard dachshunds  $P = 0.095$ ). However, the results demonstrated that LHDs and WHDs were affected twice as often ( $P < 0.001$ ) as SHDs (LHDs: 4.7%, CI<sub>95</sub>: 3.8–5.6%; WHDs: 4.1%, CI<sub>95</sub>:

3.6–4.5% and SHDs: 2.1%, CI<sub>95</sub>: 1.4–2.9, respectively). The mean age of the first diagnosis was  $4.7 \pm 2.88$  years. The most prevalent type of cataract was cortical ( $n = 144$ , 1.2%), followed by posterior polar ( $n = 52$ , 0.4%), nuclear ( $n = 39$ , 0.3%), and anterior at the suture lines ( $n = 13$ , 0.1%) and punctata ( $n = 8$ , 0.1%).

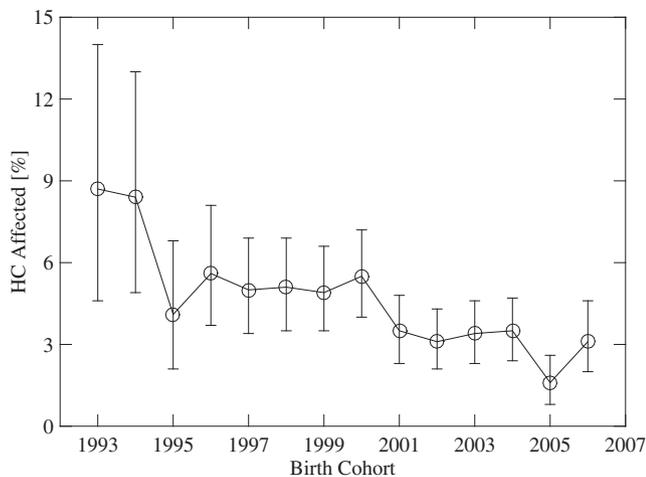
Only 0.5% (CI<sub>95</sub>: 0.4–0.6) of the examined dachshunds were affected by CC: 38 (0.5%) of all the females and 27 (0.7%) of all the males. The WHD was most frequently identified, but this was not statistically significant.

Fiberglass-like cataract was most frequently seen in WHDs ( $P < 0.001$ ) with a prevalence of 2.2% (CI<sub>95</sub>: 1.9–2.4%), affecting 99 (2.5%) of all males and 173 (2.1%) of all females. The relative risk of diagnosis of HC was 1.4 times higher (RR: 1.41, OR: 1.44,) if the dog was affected by FGC; however, this was not statistically significant ( $P = 0.172$ ).

Prominent suture lines were also seen most frequently in WHDs ( $P < 0.001$ ) and had a prevalence of 1.5%, (CI<sub>95</sub>: 1.3–1.7%) affecting 52 (1.3%) of all the males and 134 (1.6%) of all the females. A total of 10.3% (CI<sub>95</sub>: 6.3–15.5%) of the dogs that had PSLs were affected by HC. The relative risk of an HC diagnosis was 2.8 times higher (RR: 2.68, OR: 2.87) if the individual was affected by PSLs ( $P < 0.001$ ).

A total of 187 dogs (1.5%, CI<sub>95</sub>: 1.3–1.7%) were affected by PRA. This was 98 (2.5%) of all the males and 88 (1.1%) of all the females. Considering that males were examined far less often than females, they were diagnosed with PRA more than twice as often (RR: 2.31, OR: 2.35). Long-haired dachshunds were affected more frequently by PRA ( $P < 0.009$ ) than the rest and the mean age of the first diagnosis was  $5.5 \pm 3.19$  years. A total of 67 dogs (35.8%, CI<sub>95</sub>: 28.9–43.1%) diagnosed with PRA were also diagnosed with HC ( $P < 0.001$ ). The relative risk of being affected by a cataract, if diagnosed with PRA, was ten times higher (RR: 10.5, OR: 15.8) than without PRA. It is possible that some of these cataracts might have been secondary and not primary, as the information the authors had was limited to what was written in the eye certificates.

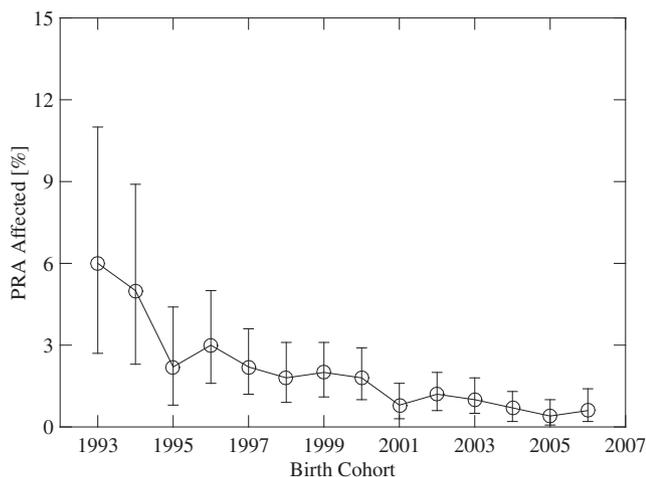
The birth cohort of 1993 had an incidence of HC of 8.7% (CI<sub>95</sub>: 4.6–14%), while dogs born in 2006 had an incidence of HC of 3.1% (CI<sub>95</sub>: 2.0–4.6%), demonstrating a significant decrease over time ( $P = 0.002$ ) (Figure 1). However, when two birth cohorts with more equal group sizes were taken from the center of the time span included in the study (1998: 5.1% and 2004: 3.5%) and were compared, the results demonstrated the decrease in incidence of HC was not statistically significant ( $P = 0.098$ ), although it showed a decreasing trend toward the younger generations. The incidence of HC, in the cohorts of 2001–2006, was between 3.1 and 3.5%. As the amount of dogs examined increased over time, the confidence intervals of the incidence per birth year decreased in favor of statistical accuracy.



**Figure 1.** Incidence and confidence intervals of hereditary cataract (HC) in birth cohorts of dachshunds in Germany from 1993 to 2006.

A significant ( $P < 0.001$ ) decrease in the incidence of PRA was found in dogs born between 1993 and 2006 (Figure 2). The incidence of the 1993 birth cohort was 6.0% (CI<sub>95</sub>: 2.7–11%) while within the cohort of 2006, this was 0.6% (CI<sub>95</sub>: 0.2–0.4%). Comparing two birth cohorts with more equal group sizes from the center of the time span demonstrated that in the group born in 1998, 1.8% of dachshunds were affected by PRA, compared to only 0.7% of the dogs born in 2004. Hence, the relative risk of PRA decreased by approximately 50% (RR: 2.17, OR: 2.74) over time, when comparing dogs born in 1998 to dogs born in 2004, which proved to be statistically significant ( $P = 0.025$ ).

There was no significant difference between  $F_s$  in groups of healthy dogs compared to the groups of dogs affected by HC or PRA within the DTK population. The



**Figure 2.** Incidence and confidence intervals of progressive retinal atrophy (PRA) in birth cohorts of dachshunds in Germany from 1993 to 2006.

average  $F$  in group 1, including dachshunds diagnosed with PRA ( $n = 111$ ), was 5.2% (min 0%, max. 26.9%). The coefficient of the comparison group 3 ( $n = 165$ ) proved to be slightly lower with 5.1%, however, not statistically significant ( $P = 0.882$ ). Group 2, which consisted of dachshunds with HC ( $n = 351$ ), had an  $F$  of 4.6% (min: 0%, max. 27.7%). The comparison group 4 ( $n = 492$ ) showed a slightly higher coefficient of 4.9%, which was also not statistically significant ( $P = 0.277$ ). An additional calculation was performed after reducing Group 2 to all dogs with a complete pedigree until the fifth generation ( $n = 315$ ), which increased the  $F$  to 5.7%.

## DISCUSSION

This is the first study to present the prevalence of a selection of HED/PIEDs spanning 13 years of eye examinations of dachshunds in Germany. It is also the first study to demonstrate the positive effect of eye examination-based breeding advice on the incidence of HC and PRA of this breed.

The focus of this study was on hereditary cataracts and progressive retinal atrophy of dachshunds in Germany, where the breed originates. The DTK excludes affected dogs from breeding because these diseases may lead to visual disability and eventually to total blindness.<sup>19</sup> The DTK required all dogs used for breeding to undergo biennial eye examinations until 2013; when the DTK felt that there was subjective evidence, the prevalence of these diseases was lower than in previous years. Based on this, the DTK opted for a single mandatory HED/PIED eye examination performed before the breeding license was issued and made additional eye examinations optional.<sup>5</sup> However, a single compulsory eye examination before the breeding license is issued carries its own risks as it could be performed before the late onset of some conditions.

Reliable genetic tests could help breeders find and exclude carriers of the known mutations for retinal disease and/or cataracts before animals reach breeding maturity. However, DNA testing could lead to confusion: It is not infallible, no responsible gene has been found for HC in dachshunds and the genetics of PRA in dachshunds have proven to be rather complex.

A mutation of the HSF4 gene is associated with cataract development in the Staffordshire bull terrier, Boston terrier, and Australian Shepherd.<sup>20–22</sup> However, the connection between HC and the HSF4 gene was excluded for dachshunds and other breeds.<sup>8</sup> In addition, three gamma-crystallins (CRYGB, CRYGC, and CRYGS),<sup>23</sup> the canine beta-crystallin,<sup>24</sup> and 17 candidate genes were also excluded from harboring mutations that could cause HC in dachshunds.<sup>25</sup> A heterogenic origin of HC cannot be ruled out at present and further research needs to be undertaken before solid breeding recommendations based on DNA tests only can be made for HC in dachshunds.

Progressive retinal atrophy and cone-rod dystrophy are collective terms for two broad forms of progressive, bilateral degenerative diseases that affect the retinal photoreceptor cells.<sup>26</sup> The authors of the present study use the general term PRA for both broad forms because this is the term used in the ECVO certificate. However, veterinarians and the general public should be aware at least two mutations for cone-rod dystrophy have been identified in dachshunds<sup>27–31</sup> and that there might be other forms of PRA that affect these dogs. An inherited, early onset cone-rod dystrophy (crd) that affected standard and miniature WHDs was described in 2007, and a mutation in the NPHP4 gene was later found to be responsible.<sup>27,31–33</sup> An autosomal recessive mode of inheritance was described, and the prevalence in miniature WHDs in the United Kingdom was reported to be 3.1%.<sup>34</sup> Studies in miniature LHDs found a second cone-rod dystrophy (cord1) with an autosomal recessive inheritance.<sup>35</sup> However, this time, a RPGRIP1 mutation was proposed as the possible cause.<sup>29</sup> Recently, the RPGRIP1 mutation has been identified in miniature SHDs and miniature WHDs in the UK.<sup>36</sup> Homozygote dogs for the RPGRIP1 mutation showed a reduction in cone-derived ERG amplitudes, although some of them had no noticeable clinical signs<sup>37</sup> and further studies proved there was a phenotype–genotype discordance.<sup>38</sup> This was explained in part by a gene mutation in chromosome-15 that was found to be responsible for causing early onset cord1 when expressed in combination with a mutation in RPGRIP1, while the mutation in RPGRIP1 on its own simply caused a late onset form or clinically unremarkable dogs.<sup>30</sup> Moreover, the heterogeneity of PRA within and between different dog breeds was further explained by a subsequent study.<sup>39</sup> There are commercially available genetic tests for dachshunds, including one for crd (NPHP4) and one for cord1 (RPGRIP1).<sup>40</sup> However, the significance of RPGRIP1 testing is questionable, as the phenotype–genotype correlation is variable.<sup>41</sup> All of this suggests that due to the heterogenic nature of PRA in dachshunds, a genetic test on its own would not be the right approach to detect all affected animals.

Until more information is available on the inheritance and genetic testing for HC and PRA in dachshunds, the authors of the present study believe that compulsory eye examinations, possibly in conjunction with genetic testing, are indicated to further decrease and prevent an increase of the incidence of both PRA and HC.

Breed screening examinations might lead to false-negative or false-positive diagnoses, as dogs with acquired cataracts (e.g., secondary to PRA or traumatic) or with PSLs could potentially be misdiagnosed as HC or vice versa, and a dog that might initially pass as unaffected could become obviously affected later in time. These are concerning possibilities although the risk of inadvertently including a dog with a potentially blinding HED in the

breeding population would arguably have a greater impact in the population than excluding an animal with a false-positive result.

The present study demonstrated that there was a significant decrease in incidence of PRA and a decreasing trend of HC in dachshunds in Germany over the time period studied. The incidence of PRA dropped from 6.0% in 1993 to 0.6% in 2006, and for HC, it decreased from 8.7% in 1993 to between 3.1% and 3.5% in the period between 2001 and 2006. These results suggest that frequent HED examinations combined with controlled breeding tactics may help reduce the incidence of HED. Therefore, it seems reasonable to not limit this approach. These findings are in agreement with previous studies on the incidence and prevalence of PHTVL/PHPV in Dobermans and the successful breeding programs to reduce this inherited problem in the breed.<sup>42</sup> Moreover, the present study demonstrates that HC and PRA in dachshunds are usually diagnosed relatively late (HC:  $4.7 \pm 2.88$  years, PRA:  $5.5 \pm 3.19$  years), which supports the recommendation to continue with regular breed screening eye examinations to at least 8–9 years of age, as long as dogs are breeding, and ideally even after they are no longer used for breeding.

The total prevalence of HC in the dachshund population of the present study was 3.9%, while the prevalence of PRA was 1.5%. The prevalence of HC and PRA in Gresky's study was higher with values of 4.6% and 1.6% respectively.<sup>7</sup> The most common location of HC in the present study was the cortex, followed by the posterior pole, while in Gresky's study, it was the anterior capsule and the posterior cortex. The ECVO forms give the user the option to use up to five terms to describe the location of a cataract including 'posterior pole' and 'cortex' but not 'posterior cortex' as used in Gresky's study. It is possible that the terms posterior pole and posterior cortex refer to the same cataract. However, due to the retrospective nature of this study, the localization of cataracts was based on what was written in the certificate and could not be specified further. Long-haired dachshunds and WHDs were found to be affected significantly more frequently by HC than SHDs. Gresky found that the WHDs were most commonly affected by HC.<sup>7</sup> A total of 4.9% of LHDs in the present study were affected with HC, more than twice as many compared to the North American population included in one study of 2005, where 2.1% of LHDs were affected by HC.<sup>19</sup>

A total of 35.8% of the dogs diagnosed with PRA in the present study were also diagnosed with HC, supporting the claims of previous authors of a correlation between cataracts and PRA.<sup>43,44</sup> It is possible that not all cataracts listed in the data were truly hereditary and that some might have been secondary to PRA or trauma. Another interesting finding was that PSLs correlated with an increased risk of HC. This might be important, because PSLs could potentially indicate a

developing HC and it would be worth to consider monitoring the affected dogs closer for potential development of HC.

An additional finding of interest was that male dachshunds of the DTK population were affected by HC and PRA significantly more often than females. Progressive retinal atrophy was seen twice as often in male dogs than in females. Gresky's study found that there were no significant differences between the sexes for HC and PRA, while a study by Mueller in 2007 confirmed a significantly higher prevalence for HC in males.<sup>7,45</sup> Further studies are warranted to investigate the etiology of the high prevalence of PRA and HC in male dachshunds in this population.

It is of interest to note that more than two-thirds of the dogs in the present study were females (68%,  $n = 8268$ ) and only one-third were males (32%,  $n = 3974$ ). DTK regulations stipulate that females may be bred from 15 months of age up until the end of their eighth year of life and that they may have a maximum of two litters within 12 months, while breeding males do not have an age limit or a limited number of matings once they have been licensed for breeding.<sup>5</sup> Some male dachshunds in the present study had a high number of offspring. The three males with the highest number of offspring had between 900 and 1150 descendants each. Unless breeding advice is based on reliable testing methods, it is possible for affected males to pass on one or more HEDs to a large number of offspring, which could increase the incidence of HC and PRA to the pre-eye testing levels of one and a half decades ago. Only dogs that were diagnosed to be affected by HC or PRA and not their offspring were excluded from breeding,<sup>5</sup> allowing the offspring to get back into the study population and potentially breed before the late onset diseases HC or PRA could have been diagnosed.

The two most common problems observed in the present study were DIST and PPMs. As these problems do not threaten vision in dachshunds, affected animals do not have to be excluded from breeding. However, DIST can lead to recurring and painful ulcers<sup>46</sup> and some forms of PPMs may lead to cataracts and/or leukoma.<sup>47</sup> It would be interesting to study the effects on the incidence of these problems when breeding mildly affected, or possibly moderately affected dogs to unaffected dogs, or breeding either of those to other affected dogs (random combinations), or breeding affected to other affected dogs. Experimental breeding would carry a significant ethical weight, although it could be helpful to prove whether breeding away from these issues would make a clinically significant difference. The exclusion of dogs with any form of PIED from the breeding pool must be carefully regulated. For example, the exclusion from breeding of descendants of dogs that are found to be affected by HC or PRA after they have been bred might contain the spread of defective alleles, but it might also drastically restrict the gene pool

by excluding homozygous, disease-free dogs from breeding. Therefore, this is not recommended.

There was no significant difference between  $F_s$  in groups of healthy dogs compared to dogs affected by HC or PRA in the present study. However, all  $F_s$  are likely to be higher than they appear, as they can be lowered by incomplete pedigrees. The  $F$  in this study was slightly lower than in other studies.<sup>7,8</sup> The authors of the present study suggest this might be due to information gaps in the pedigrees of the dogs studied or by a decreasing rate of inbreeding, as suggested in Gresky's study.<sup>7</sup>

There were limitations to the present study due to its retrospective nature, which may have influenced the results. The data of HED examinations were based on the information in the examination certificates and built the foundation for the descriptive statistics. This meant the examination data were standardized, but no additional information on individual cases or more fine-tuned anatomic localization of certain HEDs, such as some of the HCs, was available. Despite the statistical power of this study, there was a low potential for minor overreaching of conclusions.

## CONCLUSIONS

The study presents the prevalence of a selection of HED/PIEDs spanning 13 years of eye examinations of dachshunds in Germany. The findings demonstrate the positive effect of eye examination-based breeding advice on the incidence of HC and PRA of this breed. The high number of matings by a few male dogs, combined with the end of compulsory eye examinations, carries the risk of increasing the incidence of one or more HED/PIEDs over time. The findings of this study suggest the continuation of regular eye examinations by ophthalmic specialists is highly recommended in all breeding dachshunds, especially in actively breeding males. The fact that HC and PRA in dachshunds are usually diagnosed relatively late (HC:  $4.7 \pm 2.88$  years, PRA:  $5.5 \pm 3.19$  years) supports the recommendation to continue regular examinations until the age of 8 or 9 years. If subsequent voluntary/obligatory exams are not pursued, the benefit might be lost and the incidence of these HEDs may rise again.

## ACKNOWLEDGMENTS

The authors thank the German Dachshund club (DTK 1888e.V.) for providing the studbook data and the Dortmunder Kreis for providing the data of HED examinations.

## REFERENCES

1. ECVO. ECVO Manual: ECVO Scheme. European College of Veterinary Ophthalmologists, 2016; URL: <http://www.ecvo.org/inherited-eye-diseases/ecvo-manual> [accessed on 31 January 2016].

2. DOK. Dortmunder Kreis e.V. 2009; URL: <http://www.dokvet.de/> [accessed on 25 November 2013].
3. DTK. Zucht- und Eintragungsbestimmungen (regulations for breeding and registration). Deutscher Teckelklub 1888 eV. Duesburg 2011.
4. DTK. Zucht- und Eintragungsbestimmungen (regulations for breeding and registration). Deutscher Teckelklub 1888 eV. Duesburg 2007.
5. DTK. Zucht- und Eintragungsbestimmungen (regulations for breeding and registration). DTK 1888 e.V.; 2015 [updated 2013]; URL: [http://www.dtk1888.de/\\_rec/uploads/offizielles/zeb\\_2013\\_aenderung\\_markiert.pdf](http://www.dtk1888.de/_rec/uploads/offizielles/zeb_2013_aenderung_markiert.pdf) [accessed on 5 December 2015].
6. ACVO. Ocular disorders presumed to be inherited in purebred dogs. Genetics Committee of the American College of Veterinary Ophthalmologists; 2014.
7. Gresky C. *Genetische Analysen zur Vererbung der Katarakt und Progressiven Retina Atrophie beim Dackel (Genetic analysis on inheritance of Cataract and Progressive Retinal Atrophy)*. Tierärztliche Hochschule Hannover, Hannover, 2004.
8. Müller C. *Molekulargenetische Analyse der primären Katarakt beim Dackel und beim Entlebucher Semmenhund, (Molekulargenetische analysis of primary cataract in dachshunds and Entlebucher Mountain dog) [Dis]*. Tierärztliche Hochschule, Hannover, 2007.
9. ACVO. Ocular Disorders. Presumed to be inherited in purebred dogs. 5th edn: American College of Veterinary Ophthalmologists, Genetics Committee, 2010.
10. ACVO. Ocular disorders presumed to be inherited in purebred dogs. 6th edn: Genetics Committee of the American College of Veterinary Ophthalmologists, 2013.
11. ECVO. Hereditary Eye Diseases: Procedure for the Eye examination. European College of Veterinary Ophthalmologists, 2016; URL: <http://www.ecvo.org/inherited-eye-diseases/examination> [accessed on 31 January 2016].
12. ECVO, ed. European College of Veterinary Ophthalmology. Presumed inherited eye diseases. Procedure Notes, Berlin, 2010.
13. FCI. Fédération Cynologique Internationale. 1998–2010; URL: <http://fci.be/> [accessed on 11 November 2013].
14. ECVO Manual: The ECVO-certificate [database on the Internet]. European College of Veterinary Ophthalmologists. 2012. URL: <http://www.ecvo.org/inherited-eye-diseases/ecvo-manual> [accessed on 5 December 2015].
15. Bender R, Lange S, Ziegler A. Multiple testing. *Deutsche Medizinische Wochenschrift* 2007; **132** (Suppl. 1): e26–e29.
16. Dorland. Dorland's Medical Dictionary for Health Consumers. Saunders, ed.: Elsevier; 2007.
17. Falconer DS, Mackay TFC. *Introduction to Quantitative Genetics*, 4th Edition. Longman Group Ltd, Harlow, 1996.
18. Henderson CR. A simple method for computing the inverse of a numerator relationship matrix used in prediction of breeding values. *Biometrics* 1976; **32**: 69–83.
19. Gelatt KN, Mackay EO. Prevalence of primary breed-related cataracts in the dog in North America. *Veterinary Ophthalmology* 2005; **8**: 101–111.
20. Mellersh CS, Graves KT, McLaughlin B *et al.* Mutation in HSF4 associated with early but not late-onset hereditary cataract in the Boston Terrier. *Journal of Heredity* 2007; **98**: 531–533.
21. Mellersh CS, Pettitt L, Forman OP *et al.* Identification of mutations in HSF4 in dogs of three different breeds with hereditary cataracts. *Veterinary Ophthalmology* 2006; **9**: 369–378.
22. Mellersh CS, McLaughlin B, Ahonen S *et al.* Mutation in HSF4 is associated with hereditary cataract in the Australian Shepherd. *Veterinary Ophthalmology* 2009; **12**: 372–378.
23. Muller C, Wohlke A, Distl O. Evaluation of three canine gamma-crystallins (CRYGB, CRYGC, and CRYGS) as candidates for hereditary cataracts in the dachshund. *Molecular Vision* 2007; **13**: 125–132.
24. Muller C, Distl O. Linkage and association analyses of intragenic SNPs in the canine beta-crystallin genes CRYBB1, CRYBB2, CRYBB3, CRYBA1 and CRYBA4 with primary cataracts in wire-haired dachshunds. *Animal Genetics* 2008; **39**: 87–88.
25. Muller C, Distl O. Scanning 17 candidate genes for association with primary cataracts in the wire-haired Dachshund. *The Veterinary Journal* 2009; **182**: 342–345.
26. Mellersh CS. The genetics of eye disorders in the dog. *Canine Genetics and Epidemiology* 2014; **1**: 1–14.
27. Wiik AC, Wade C, Biagi T *et al.* A deletion in nephronophthisis 4 (NPHP4) is associated with recessive cone-rod dystrophy in standard wire-haired dachshund. *Genome Research* 2008; **18**: 1415–1421.
28. Wiik AC, Ropstad EO, Bjerkas E *et al.* A study of candidate genes for day blindness in the standard wire haired dachshund. *BMC Veterinary Research* 2008; **4**: 1–7.
29. Mellersh CS, Boursnell ME, Pettitt L *et al.* Canine RPGRIP1 mutation establishes cone-rod dystrophy in miniature longhaired dachshunds as a homologue of human Leber congenital amaurosis. *Genomics* 2006; **88**: 293–301.
30. Miyadera K, Kato K, Boursnell M *et al.* Genome-wide association study in RPGRIP1(-/-) dogs identifies a modifier locus that determines the onset of retinal degeneration. *Mammalian Genome* 2012; **23**: 212–223.
31. Palanova A, Schrofflova D, Pribanova M *et al.* Analysis of a deletion in the nephronophthisis 4 gene in different dog breeds. *Veterinary Ophthalmology* 2014; **17**: 76–78.
32. Ropstad EO, Bjerkas E, Narfstrom K. Electroretinographic findings in the Standard Wire Haired Dachshund with inherited early onset cone-rod dystrophy. *Documenta Ophthalmologica* 2007; **114**: 27–36.
33. Ropstad EO, Bjerkas E, Narfstrom K. Clinical findings in early onset cone-rod dystrophy in the Standard Wire-haired Dachshund. *Veterinary Ophthalmology* 2007; **10**: 69–75.
34. AHT. Cone-rod dystrophy form of progressive retinal atrophy in the wire-haired dachshund (miniature and standard). 2013; URL: <http://www.aht.org.uk/> [accessed on 18 October 2015].
35. Turney C, Chong NHV. Pathological and electrophysiological features of a canine cone-rod dystrophy in the miniature longhaired dachshund. *Investigative Ophthalmology & Visual Science* 2007; **48**: 4240–4249.
36. AHT. Progressive retinal atrophy in miniature long-haired, miniature smooth-haired and miniature wire-haired dachshunds. Newmarket, Suffolk 2015; URL: [http://www.aht.org.uk/cms-display/genetics\\_dachpra.html](http://www.aht.org.uk/cms-display/genetics_dachpra.html) [accessed on 18 October 2015].
37. Busse C, Barnett KC, Mellersh CS *et al.* Ophthalmic and cone derived electrodiagnostic findings in outbred Miniature Long-haired Dachshunds homozygous for a RPGRIP1 mutation. *Veterinary Ophthalmology* 2011; **14**: 146–152.
38. Miyadera K, Kato K, Aguirre-Hernandez J *et al.* Phenotypic variation and genotype-phenotype discordance in canine cone-rod dystrophy with an RPGRIP1 mutation. *Molecular Vision* 2009; **15**: 2287–2305.
39. Downs LM, Hitti R, Pregolato S *et al.* Genetic screening for PRA-associated mutations in multiple dog breeds shows that PRA is heterogeneous within and between breeds. *Veterinary Ophthalmology* 2014; **17**: 126–130.

40. AHT. Animal Health Trust. Suffolk, Newmarket 2013 [updated Stand: 05.12.2013]; URL: <http://www.aht.org.uk/> [accessed on 05 December 2013].
41. Aguirre G, ed. Control of hereditary eye diseases in dog and cat populations. ECVO Conference; Trieste, University of Pennsylvania, 2012.
42. Stades FC, Boeve MH, van den Brom WE *et al.* The incidence of PHTVL/PHPV in Doberman and the results of breeding rules. *Veterinary Quarterly* 1991; **13**: 24–29.
43. Zigler JS, Hess HH. Cataracts in the Royal College of Surgeons rat: evidence for initiation by lipid peroxidation products. *Experimental Eye Research* 1985; **41**: 67–76.
44. Zigler JS Jr, Bodaness RS, Gery I *et al.* Effects of lipid peroxidation products on the rat lens in organ culture: a possible mechanism of cataract initiation in retinal degenerative disease. *Archives of Biochemistry and Biophysics* 1983; **225**: 149–156.
45. Muller C, Hamann H, Brahm R *et al.* Analysis of systematic and genetic effects on the prevalence of different types of primary lens opacifications in the wild-boar-colored wirehaired Dachshund. *Berliner Münchner Tierärztliche Wochenschrift* 2008; **121**: 286–291.
46. Bedford PG. Eyelashes and adventitious cilia as causes of corneal irritation. *Journal of Small Animal Practice* 1971; **12**: 11–17.
47. Roberts SR, Bistner SI. Persistent pupillary membrane in Basenji dogs. *Journal of the American Veterinary Medical Association* 1968; **153**: 533–542.