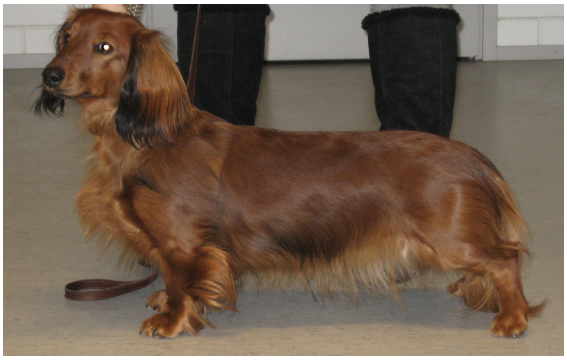
Progressing a scoring scheme for intervertebral disc calcification screening in Dachshund dogs



Master of Philosophy (MPhil) Thesis

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Abstract

Intervertebral disc disease (IVDD) is a major health problem in Dachshunds. Of all the dog breeds they have the highest incidence of IVDD, owing to their chrondrodystrophy and subsequent accelerated intervertebral disc (IVD) degeneration. Degenerated IVDs are predisposed to herniation, resulting in spinal cord injury and commonly paralysis. Late-stage IVD degeneration can include dystrophic calcification, and this calcification may be detected on spinal radiographs (radiographically detectable intervertebral disc calcification [RDIDC]). IVDD and RDIDC are highly heritable in Dachshunds, with RDIDC scores at young adult age being a strong predictor of clinical IVDD occurrence later in life. A screening program was developed whereby potential breeding candidates undergo spinal radiography and scoring for RDIDC, with the aim of reducing the incidence of IVDD through selective breeding.

Despite the existence of a large body of literature around IVDD in Dachshunds, including a solid scientific basis for the development of the radiographic screening tool, several deficiencies and areas for ongoing research were identified and guided this project. Widespread global awareness and application of the screening program was lacking. Therefore, an extensive appraisal of the literature was performed resulting in the paper 'Radiographic scoring for intervertebral disc calcification in the Dachshund', which is available in the *Veterinary Journal*.

For a test to be useful it must be precise, and the scorer variability (precision) for RDIDC scoring had not been evaluated. Accordingly, the within-scorer (repeatability) and between-scorer (reproducibility) variability of RDIDC scoring was estimated using five scorers with varying levels of prior experience, both at the individual IVD level and at the whole dog level for breeding classification purposes. Overall, RDIDC scoring was found to be highly consistent within scorers, with increased precision achieved by scorers with greater experience. However, unique individual

scorer patterns were identified as a factor. These results supported the ongoing use of the screening program, and were published in *Preventive Veterinary Medicine*.

Investigation of the utility of alternate diagnostic imaging modalities, namely computed tomography (CT) and magnetic resonance imaging (MRI), for scoring IVD calcification in Dachshunds had not previously been undertaken. The first step in evaluating these modalities for potential use was to assess scorer agreement for CT and MRI, and compare these results with radiography (i.e. RDIDC scoring). Supporting the results of prior work, radiography was identified as a highly precise test with repeatability and reproducibility estimates that were greater than for CT and MRI, likely attributable to scorer familiarity with the modality and RDIDC scoring. Again, increased scorer experience corresponded with greater RDIDC scoring precision. Despite these findings, CT identified substantially higher overall numbers of IVD calcifications than the other modalities, and further analysis of this data to examine the accuracy of the various modalities is warranted.

Despite the work achieved through this project, ongoing research is needed. Additional experiments are planned, including analysis of the potential effect of instruction and training on RDIDC scorer subjectivity, and an epidemiological study of the Australian Dachshund population.

Abbreviations

IVD – intervertebral disc

IVDD – intervertebral disc disease

RDIDC – radiographically detectable intervertebral disc calcification

CT – computed tomography

MRI – magnetic resonance imaging

Thesis Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

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Acknowledgements

This Master of Philosophy program was undertaken over a prolonged course, including part-time and remote candidature necessitated by concurrent completion of a 3-year veterinary diagnostic imaging residency and subsequent specialty board examinations in North America. All participants, including supervisors and co-authors, provided unwavering support despite the long-distance nature of our working relationships and extended timeframe, allowing me to see this program and thesis to completion. For this I am sincerely grateful.

Contextual Statement

Intervertebral discs (IVDs) are located between the vertebral bodies in the spine, being fibrocartilaginous hydroelastic cushions that act as shock absorbers. Intervertebral disc disease (IVDD) is a condition observed in dogs as well as other species including humans. IVDD encompasses a spectrum of potential manifestations including disc degeneration with or without herniation. When material inside a disc displaces (herniates) into the spinal canal, it can compress and injure the spinal cord resulting in pain and neurologic dysfunction, potentially leading to complete paralysis and irreversible spinal cord damage. Dehydration of an intervertebral disc (IVD) can occur normally with ageing, which limits its ability to transfer and distribute loads between vertebrae; however, IVD degeneration can also occur prematurely and rapidly in chondrodystrophic breeds of dog such as the Dachshund. In chondrodystrophy, the IVDs undergo chondroid metaplasia resulting in the early maturation and degeneration, and in the late stage, dystrophic calcification. Degenerated IVDs are predisposed to herniate under minimal stress. Compared to the wider canine population, Dachshunds have the highest incidence (16-25% vs. 2%) and relatively risk (10-12 times higher than other breeds) of clinical IVDD.

Intervertebral disc calcification is highly heritable in Dachshunds. Further, Dachshunds with increased numbers of IVD calcifications in their spine at 2-3 years of age are at greater risk of clinical IVDD than those without calcifications at this age. IVD calcifications in Dachshunds are at their highest number between 24 and 27 months of age, as detected by radiography. Thus, radiographic spinal screening is recommended to be performed at this age. A screening scheme, which originated in Scandinavia around 15 years ago, is in place to assist Dachshund breeders to select appropriate breeding candidates, with the aim of reducing the incidence of IVDD in the breed.

The scheme involves scoring each dog for the total number of radiographically detectable intervertebral disc calcifications (RDIDC) within the spine (from 0 up to a maximum of 26 possible IVDs [excluding tail IVDs]). Current recommendations, based on research findings, are to preferentially breed from dogs with a RDIDC score of ≤ 2, use dogs with a score of 3 or 4 judiciously, and excluded dogs with ≥ 5 RDIDCs from breeding. The sensitivity and specificity of radiography for detecting IVD calcification is 0.6 and 1.0, respectively, when histopathology is used as the gold standard. However, scorer variability in scoring radiographs for RDIDC had not been verified. Further, more advanced diagnostic imaging modalities that are routinely used to image the canine spine in veterinary practice, including computed tomography (CT) and magnetic resonance imaging (MRI), have not been assessed for their potential utility in scoring Dachshund spines for IVD calcification. Given that CT and MRI are cross-sectional modalities with superior contrast resolution compared to radiography, it might be anticipated that they have improved precision and accuracy as screening tools.

Therefore, the aims of this Master's research were to (i) undertake a review of the literature around IVDD and RDIDC scoring in the Dachshund and develop a summary report that would be widely available, (ii) determine the precision of scoring Dachshund spines for RDIDC (i.e. within-scorer variability [repeatability] and between-scorer variability [reproducibility]), and (iii) compare the precision, robustness and agreement between three diagnostic imaging modalities (radiography, CT and MRI) for screening IVD calcification in Dachshund spines. The overarching aim was to progress and optimise the established screening scheme.

Chapter 1: Literature Review Publication

Review: Radiographic scoring for intervertebral disc calcification in the Dachshund

Statement of Authorship

Title of Paper	**Review: Radiographic scoring for intervertebral disc calcification in the Dachshund**
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Principal Author

Name of Principal Author (Candidate)	Alana J Rosenblatt
Contribution to the Paper	Perform comprehensive literature search and review Writing of the manuscript, including revisions Addressing peer-review comments and revising manuscript
Overall percentage (%)	90%
Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.
Signature	Date 07/02/17

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Cynthia D Bottema		
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Review

Radiographic scoring for intervertebral disc calcification in the Dachshund

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ABSTRACT

Intervertebral disc disease is a common, painful and debilitating neurological condition of dogs, causing substantial morbidity and mortality. The Dachshund is particularly susceptible to this disorder. The goal of this article is not to duplicate previously published reviews on canine intervertebral disc degeneration and degenerative diseases. Rather, the aims are threefold: (1) to reflect on selected clinical and pathophysiological aspects of intervertebral disc degeneration and disc disease that are pertinent to the Dachshund breed; (2) to review a radiographic spinal scoring scheme developed to reduce the prevalence of intervertebral disc disease in Dachshunds; and (3) to suggest further areas of research to improve upon the currently established scoring scheme in an attempt to address this breed's greatest health problem.

Introduction

The function of the intervertebral disc (IVD) is to provide stability, mobility and flexibility to the vertebral column, and to absorb shock and disperse pressure (Hansen, 1952; Bray and Burbidge, 1998a; Bergknut et al., 2013). The highly specialized central nucleus pulposus (NP) is the dynamic, functional portion of the disc due to its high water content, allowing it to act as a hydraulic cushion during loading, while the outer collagenous annulus fibrosus (AF) provides the majority of the disc's strength and tenacity (Hansen, 1952, 1959; Ghosh et al., 1977).

Degeneration of the IVD is a complex, self-perpetuating process, and many features seen in chondrodystrophic dogs such as the Dachshund vary from those that occur in non-chondrodystrophic breeds (Bray and Burbidge, 1998b). Chondroid metaplasia typically begins at a very young age, and is characterized by rapid maturation in which newly formed cells tend to be chondrocyte-like rather than of native notochordal origin, so that by 1 year of age the majority of the formerly gelatinous NP may consist of hyaline cartilage (Hansen, 1951, 1952, 1959, 1964; Goggin et al., 1970; Braund et al., 1975; Gage, 1975; Ghosh et al., 1976; Priester, 1976; Bray and Burbidge, 1998b; Jensen and Christensen, 2000a; Brisson et al., 2004; Parker et al., 2009; Bergknut et al., 2013; Smolders et al., 2013). The hardened, and often calcified, NP is now predisposed to rupture through the AF under minimal stress, with explosive dorsal displacement of disc material into the vertebral canal (Hansen type I disc disease) (Hansen, 1951, 1952; Ghosh et al., 1977; Seiler et al., 2011). Readers are referred to the literature on canine IVD disease (IVDD), such as recent articles by Brisson (2010), Bergknut et al. (2013), and Smolders et al. (2013), for further revision of normal IVD anatomy and function, and the pathophysiology of disc degeneration.

The estimated lifetime prevalence of IVDD in the general canine population is 2–3.5% (Simpson, 1992; Bergknut et al., 2012). The prevalence is much higher in certain breeds, however, and is by far the highest in the Dachshund (Bergknut et al., 2012). Nineteen to 25% of Dachshunds are expected to experience clinical signs of IVD herniation in their lifetime, with a relative risk of disease 9.9–12.6 times that of the general dog population (Goggin et al., 1970; Gage, 1975; Priester, 1976; Ball et al., 1982; Simpson, 1992; Bergknut et al., 2012). Further, Dachshunds with IVD herniation constitute 45–84.6% of all acute IVDD cases worked-up in veterinary hospitals (Knecht, 1970; Brown et al., 1977; Hoerlein, 1979; Scott, 1997; Necas, 1999; Aikawa et al., 2012b). The peak age of onset occurs between 3 and 7 years in chondrodystophic breeds, with the peak in Dachshunds between 4 and 6 years of age (Knecht, 1970; Gage, 1975; Priester, 1976; Brown et al., 1977; Olby et al., 2003; Newcomb et al., 2011; Aikawa et al., 2012a, 2012b). The vast majority of all disc herniations occur between T11 and L3 (Hansen, 1951, 1952; Gage, 1975; Brown et al., 1977; Hoerlein, 1979; Scott, 1997; Necas, 1999; Olby et al., 2003; Ruddle et al., 2006; Brisson et al., 2011; Newcomb et al., 2011; Aikawa et al., 2012a, 2012b).

A presumptive diagnosis of IVDD, based on signalment, history, clinical presentation and neurologic examination, can be confirmed with diagnostic imaging and/or at surgery. Multiple imaging techniques are currently available to diagnose and localize canine IVDD. For decades, myelography was the standard imaging technique and is still adequate for diagnosis when magnetic reso-

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nance imaging (MRI) and computed tomography (CT) are unavailable (Robertson and Thrall, 2011). However, myelography is an invasive technique with well-documented side effects (i.e. seizures or exacerbation of existing neurological signs) due to intra-thecal injection of neurotoxic contrast material (Butterworth and Gibbs, 1992; Lewis and Hosgood, 1992; Sande, 1992; Barone et al., 2002; da Costa et al., 2011). Compared with myelography, CT provides superior contrast resolution, image-reformatting capabilities, and short examination times (Hecht et al., 2009). Tomographic imaging also eliminates problems with superimposition.

Although non-contrast CT scans of the spine are accurate for the diagnosis and localization of mineralized discopathies in chondrodystrophic breeds (Olby et al., 2000; Sharp and Wheeler, 2005; Hecht et al., 2009; Israel et al., 2009; Dennison et al., 2010; Lim et al., 2010; Seiler et al., 2011), CT myelography is the most sensitive CT technique for identification of extradural compression of the spinal cord, and is often required for the diagnosis of non-mineralized disc displacements (Sharp et al., 1995; Dennison et al., 2010; Newcomb et al., 2011). The most recent addition to the spinal imaging repertoire, MRI is a noninvasive, safe, and accurate modality for diagnosing canine IVDD (Levitski et al., 1999). When high-field MRI is available, it is considered the optimal modality for imaging the spine (Dennis, 2011; Robertson and Thrall, 2011), because MRI offers superior tissue contrast and multiplanar imaging capability (Dennis, 2011), as well as direct visualization of lesions affecting the spinal cord parenchyma (Sharp and Wheeler, 2005).

The prognosis for dogs with IVDD is influenced by many factors, especially the degree of sensorimotor loss on presentation (presence or absence of deep nociception is considered the most important prognostic indicator). Non-ambulatory chondrodystrophic or small-breed dogs that retain deep nociception before decompressive surgery have reported recovery rates in the range of 86–96% (Gambardella, 1980; Scott, 1997; Necas, 1999; Davis and Brown, 2002; Ferreira et al., 2002; Brisson et al., 2004; Ruddle et al., 2006; Bush et al., 2007), compared to 50% of paraplegic dogs with loss of deep nociception (Gambardella, 1980; Aikawa et al., 2012a). Up to 44% of dogs have recurrence of clinical signs following decompressive thoracolumbar surgery, with 6.4–12.7% of cases requiring additional operations (Funkquist, 1970; Brown et al., 1977; Scott, 1997; Dhupa et al., 1999; Necas, 1999; Brisson et al., 2004, 2011; Mayhew et al., 2004; Aikawa et al., 2012b). Furthermore, while many dogs may go on to walk post-surgery, a significant number (20–25% of chondrodystrophic dogs) retain gait and neurological deficits, including urinary and/or fecal incontinence, and self-mutilation (Scott, 1997; Olby et al., 2003; Aikawa et al., 2012a).

In two recent retrospective studies, approximately 15% of dogs did not achieve good long-term functional outcome after surgical decompression (Bull et al., 2008; Aikawa et al., 2012a). Therefore, IVDD leads to shortened duration and quality of life for a number of affected dogs, sometimes even after expensive surgical intervention. Moreover, not all owners proceed with treatment for their dog with IVDD due to financial constraints or the inability to nurse a pet with residual neurologic deficits long term, and dogs may be euthanized because of this condition.

Relationship between intervertebral disc calcification and intervertebral disc herniation in the Dachshund

As early as 1951, Hansen postulated that there might be a relationship between the constitution of chondrodystrophic breeds of dog, the development of their IVDs, and subsequent disc herniation. In her inaugural dissertation, Havranek-Balzaretti (1980) reported that in a prospective study of 209 Dachshunds aged 12–18 months, 79% with radiographically detectable IVD calcification (RDIDC) showed clinical signs of IVDD later in life, whereas dogs without RDIDC were free of signs of the disease. Some of the dogs in that study were subsequently bred to each other, and their offspring evaluated at 12 months of age. The results were convincing: if both parents had been free of RDIDC, only 30% of the offspring had RDIDC at 12 months of age. Conversely, if one parent was free of IVD calcification but was mated to a dog with calcifications, 56% of the offspring had RDIDC, and if both parents had calcifications, 83% of the offspring also had RDIDCs. Thus, it was shown that dogs with RDIDC were more likely to develop disc herniation during their lifetime than those without RDIDC, and that RDIDC is heritable.

Over subsequent decades, researchers in Scandinavia further investigated the relationship between RDIDC and the occurrence of clinical IVDD. Surveying 16.1% of all registered Norwegian Dachshunds, Stigen (1991) estimated a 23.5% prevalence rate of RDIDC in 327 clinically normal, 12- to 18-month-old dogs. Affected dogs had a mean of 2.3 calcified discs, identified throughout all regions of the spine but most frequently in the caudal thoracic vertebral column. The occurrence of RDIDC was variable between different coat and size varieties; as these traits are genetically controlled (Parker et al., 2010), a genetic basis for the development of RDIDC is further supported. Numerous studies suggest that male and female Dachshunds are equally affected with RDIDC (Priester, 1976; Ball et al., 1982; Stigen, 1991; Jensen and Ersboll, 2000b; Jensen, 2001; Lappalainen et al., 2001; Rohdin et al., 2010).

A review of the radiographs of 21 clinically normal stud dogs, aged between 4.9 and 13.2 years, revealed an almost doubled relative risk for having RDIDC compared with previously studied 1-year-old Dachshunds (mean number of RDIDC per dog, 3.7 vs. 2.3 in the younger cohort) (Stigen, 1991, 1995). These results, which are consistent with findings from a pathologic study of chondrodystrophic dog discs (Hansen, 1952), suggest that an increase in RDIDC occurs after 1 year of age, and indicate that discs not visibly calcified in a 1-year-old Dachshund can subsequently undergo calcification (Stigen, 1995). However, Stigen's findings were in contrast to those of Havranek-Balzaretti (1980) who reported that within an adult population, the proportion of dogs with RDIDC was fairly constant. Thus, the occurrence of IVD calcification beyond 1 year of age was still uncertain.

Follow-up radiographs were performed in 115/327 1-year-old dogs (Stigen, 1991) at 5 years of age (Stigen, 1996). RDIDC was identified in 57.4% of 5-year-old dogs, with a mean of 3.2 per dog. Although 97% of the dogs that had RDIDC at 1 year of age retained disc calcifications, interestingly, 31.5% of calcified discs were no longer calcified 4 years later, and 70.1% of the calcified discs in the 5-year-old dogs had not been calcified prior. As the dogs aged, only 8.6% of dogs without RDIDC at 1 year of age developed signs of spinal disease over the 4 year interval, compared with 35.3% of the dogs in which calcified discs had been identified, representing a four times greater risk for IVDD.

Spinal radiographs of 124 Finnish miniature Dachshunds were assessed for RDIDC by another group of investigators (Lappalainen et al., 2001). A very large proportion of the dogs had RDIDC (75.9% of longhaired and 86.7% of wirehaired variants). These investigators noted that the values were higher than in previous studies (Havranek-Balzaretti, 1980; Stigen, 1996), postulating that the varied incidence of RDIDC seen with different coat types and sizes of dog (Stigen, 1991) was likely the reason for this disparity. The number and distribution of RDIDCs throughout the spine was similar to previous studies (Stigen, 1991, 1996). Clinical signs of IVDD (as reported by owners) occurred in 17.9–20% of dogs with RDIDC, lower than previously reported (Havranek-Balzaretti, 1980; Stigen, 1996). The authors recognized that the mean age of the dogs in their study was only 4 years while the peak age of clinical IVDD is 4–6 years (Gage, 1975; Priester, 1976); therefore, the incidence rate reported was not only probably lower than expected but also suggests that other factors besides IVD calcification may influence the development of IVDD (Lappalainen et al., 2001). Importantly, only 1/25 dogs

without RDIDC (4%) showed clinical signs of IVDD (Lappalainen et al., 2001).

Another study investigated the optimum age for conducting plain spinal radiographs to evaluate Dachshunds for RDIDC. Using serial radiographs of 40 Dachshunds, obtained at intervals between 6 and 24 months of age with follow-up in 12 dogs at 3–4 years of age, Jensen and Arnbjerg (2001) determined that the number of dogs affected and number of RDIDC reached a steady level at 24–27 months of age. Numbers of RDIDC in some high prevalence families of dogs were seen to decrease after 2 years of age due to the disappearance of previously identified calcifications, apparently asymptomatically in most cases (Jensen, 2001). As a consequence, Jensen and Arnbjerg (2001) recommended performing spinal radiography at 24–30 months of age to examine Dachshunds for RDIDC, particularly when using the information for heritability studies and selective breeding.

By examining eight families of wirehaired Dachshunds, where each family included both parents and a minimum of three offspring from each litter, Jensen and Christensen (2000a) demonstrated a statistically significant, high heritability estimate of 0.46–0.87 for RDIDC based on the sire–offspring relationship. Even higher estimates were obtained for the dam–offspring relationship, suggesting a maternal litter effect on the number of RDIDC in the progeny. However, the sire–offspring relationship gives a better estimate, as environmental litter effects do not influence the relationship. High heritability means that the occurrence of RDIDC should respond well to selective breeding, and that heritability estimates could be improved based on the breeding values of many relatives (Jensen and Christensen, 2000a). In that study, if both parents had RDIDC, 91% of the offspring had calcifications, whereas only 44% were affected if only one of the parents had RDIDC. Again, this supports the theory that IVD calcification is hereditary.

Recently, the association between RDIDC and disc herniation was quantified in a longitudinal study of 61 Dachshunds that had been screened for RDIDC at 2 years of age (Jensen et al., 2008). Historical data regarding occurrence of IVD herniation were collected by way of an owner-completed questionnaire. Dogs were considered to have a history of IVD herniation only when a veterinary practitioner had made the diagnosis, and the dog had been euthanized or treated on the basis of the diagnosis. Exact criteria applied in achieving the diagnosis were not investigated, however. Twenty-two (36%) of the 61 dogs had a history of IVD herniation by 8 years of age. RDIDC at 2 years of age was a significant predictor of disc herniation: in dogs with ≥9 calcifications, 88% had subsequent IVD herniation, and of those with 5–8 calcifications, 63% had IVD herniation. Comparatively, in dogs with ≤2 calcifications at 2 years of age, IVD herniation was only reported in 11.5%, and only 8% of dogs without RDIDC subsequently developed IVD herniation. Risk of euthanasia for IVD herniation was significantly higher in dogs with >4 calcified discs (37%) than those with ≤4 calcifications (4.8%). Other authors have also found that recurrence of IVD herniation is more frequent in dogs with radiographic evidence of disc mineralization at the time of first surgery, and that recurrence is more likely to occur with a disc that was mineralized (Mayhew et al., 2004; Brisson et al., 2011).

Rohdin et al. (2010) conducted a retrospective study of RDIDC in 100 Dachshunds surgically treated for disc herniation. The population of dogs in their study was suitably representative of Dachshunds with IVDD, being of variable age (mean, 6.17 years) and including all size and hair coat varieties. Spinal radiographs of these dogs were reviewed for the presence of RDIDC, and the number and localization of calcifications noted. A bimodal distribution of RDIDC, with a small peak at the cervico-thoracic junction, and a larger peak at the thoracolumbar junction, was similar to previous findings (Stigen, 1996). The collected data were then correlated with surgically confirmed extruded discs. It was found that extrusions occur with similar frequency in discs with and without RDIDC, and that IVD herniation requiring surgery occurred in the absence of any RDIDC in 13% of the dogs in this study group. However, the overall frequency of RDIDC was higher in this Dachshund population (87% total, and 47% with ≥5 RDIDC) compared to previous studies (Stigen, 1991, 1995, 1996; Jensen and Christensen, 2000a; Jensen and Ersboll, 2000b; Lappalainen et al., 2001), leading the authors to surmise that RDIDC, being a sign of severe disc degeneration, is a serious risk factor for developing IVD herniation. Five dogs who had two thoracolumbar spine surgeries performed on separate occasions had a much higher mean RDIDC score than all other dogs, again suggesting that RDIDC is a risk factor for developing disc extrusion. The study by Rohdin et al. (2010) also showed that although dogs without RDIDC can develop disc extrusion, they are at a lower risk, with that category representing only 13% of cases. Nevertheless, Rohdin et al. (2010) did reasonably suggest that further studies should be conducted to demonstrate if selection of breeding dogs based on RDIDC score will indeed reduce the incidence of disc herniation in the breed. They also noted that only a portion of IVD calcification present will be detected radiographically, thereby underestimating the real extent of disc degeneration. Certainly, the absence of RDIDC does not mean that a disc is not degenerated nor calcified (Stigen and Kolbjornsen, 2007). The need for an optimized screening tool for use in live dogs is apparent.

Screening for intervertebral disc calcification

Currently, RDIDC is the only readily available measure for predisposition to clinical IVDD. RDIDC should be rated by the total number of calcified discs within the vertebral column (i.e. a continuous numerical score between 0 and 26), as this parameter gives the strongest heritability estimate (Jensen and Christensen, 2000a) and is the better predictor of future IVD herniation compared with the number of RDIDC between T10 and L3 (Jensen et al., 2008). These findings, in addition to the high heritability of RDIDC, means that selective breeding against disc calcification in 2-year-old Dachshunds may effectively reduce the occurrence of IVD herniation (Jensen and Christensen, 2000a; Jensen et al., 2008). A number of Nordic countries, including Denmark, Norway and Finland, have initiated radiographic screening programs for RDIDC in Dachshunds aged 24–42 months (Rohdin et al., 2010; Mogensen et al., 2011). The Danish Dachshund Club recommends breeding with dogs that have ≤2 RDIDCs, excluding those with ≥5 calcifications, and using dogs with a score of 3 or 4 judiciously (Mogensen et al., 2011); these recommendations are supported by experimental findings (Jensen et al., 2008). A new initiative by the Danish Dachshund Club is the calculation of breeding values for individual dogs from available information of all animals in a given pedigree (Mogensen et al., 2011)[1]. Only dogs with a breeding value above the breed average are recommended for breeding.

To date, IVD calcification screening has only been performed using conventional radiography, arguably the most practical, available, and affordable method for the identification of disc calcification in live dogs (Stigen, 1996; Stigen and Kolbjornsen, 2007). It is imperative that the radiographic technique is standardized to augment the detection of disc calcifications, so the whole spine should be radiographed with the dog under sedation or general anesthesia to achieve excellent, repeatable positioning, and radiographic exposure should be optimized (Dennis, 1987; Lappalainen et al., 2001; Sharp and Wheeler, 2005; Jensen et al., 2008). Dogs should be placed in lateral recumbency, and at least five lateral projections made, covering the vertebral column from C2 to S3 and centered on each spinal region

[1] Dansk Gravhundeklub (Danish Dachshund Club), 2013. http://www.dgk.dk (accessed 30 December 2013).

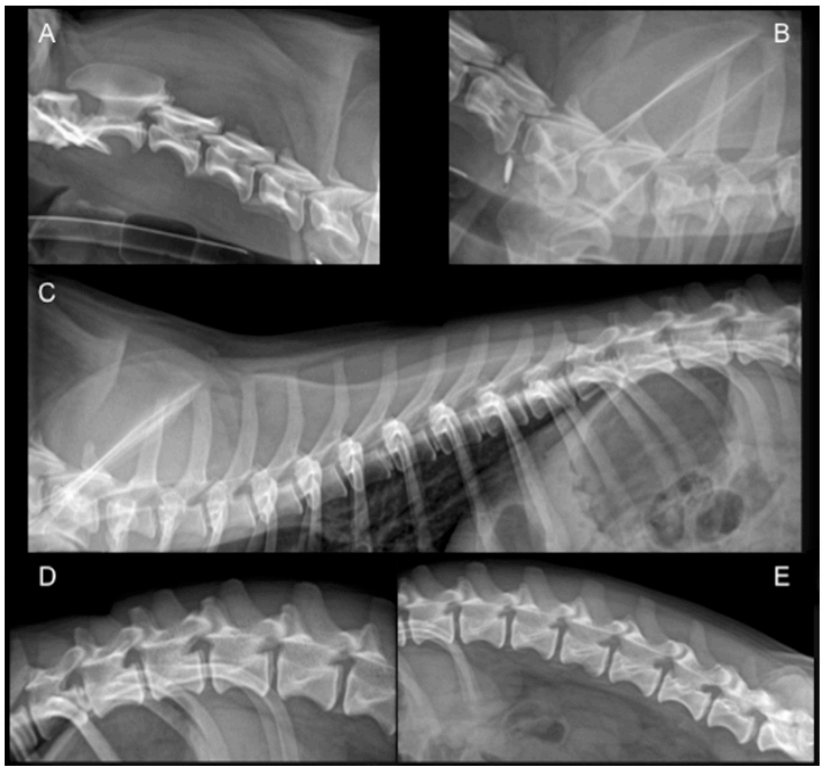

Fig. 1. Five lateral spinal radiographs of a Dachshund positioned in right lateral recumbency, obtained for scoring of intervertebral disc calcification. The radiographic beam is centered on and collimated for (A) the cervical spine [C1-C6], (B) the cervicothoracic spine junction [C5-T2], (C) the thoracic spine [C7-L1], (D) the thoracolumbar spine junction [T11-L3], and (E) the lumbar spine [L1-S1]. The dog is under general anesthesia (endotracheal tube in place).

with appropriate beam collimation (Fig. 1) (Jensen and Arnbjerg, 2001; Sharp and Wheeler, 2005; Jensen et al., 2008). Although this cannot be considered a completely benign procedure, general anesthesia poses minimal-to-nil risk in a systemically healthy dog (Bille et al., 2012). Further, disc herniation caused by manual manipulation of the spine (e.g. during radiography performed under general anesthesia) may be possible but has not been reported in clinically normal dogs to the authors' knowledge, and is rarely reported in people with pre-existing disc disease (Tamburrelli et al., 2011; Yao et al., 2013). However, in dogs with acute spinal injury, manipulations while under general anesthesia are not advised because of the risk of exacerbating neural damage (Sharp and Wheeler, 2005). The moderate financial cost to a breeder for having screening spinal radiographs performed is also an aspect to be considered, although these costs are likely much less than the potential cost of IVDD treatment for one or many dogs within their lines, and the potential long-term benefit to future generations of Dachshund dogs and their owners would seem to outweigh the minimal risk to individual animals and cost to breeders.

When using histopathology as the best reference standard, radiography has a sensitivity of 0.6 and specificity of 1.0 for the identification of IVD calcification in Dachshunds (Stigen and Kolbjornsen, 2007). Of discs that were identified as calcified only on histological examination, 91.3% had a slight degree of calcification, indicating that radiography is insensitive in detecting minor calcifications in particular (Figs. 2 and 3). Further, two dogs in this study had a total absence of RDIDC but were found to have calcification of many discs histologically. Therefore, a radiographic score of zero is not a guarantee that a dog is free from IVD calcification, and radiographic studies are far less sensitive than pathologic studies for the identification of IVD calcification (Hansen, 1952; Stigen, 1991; Stigen and Kolbjornsen, 2007). However, the focus on using radiography as a screening tool is to provide information that can be utilized as part of a breeding program, and as such, must be applicable to live dogs.

The threshold for detection of degenerative changes in an IVD will depend on the imaging method used. The last stage of disc degeneration (i.e. calcification) would be best detected with CT (Modic et al., 1988), which can be used in live dogs to detect discs with only a slight degree of calcification (perhaps representing up to 67% of affected discs) (Stigen and Kolbjornsen, 2007). We further suggest that the threshold for radiographic scoring could be redefined using CT as a superior standard, thus increasing the accuracy of radiographic spinal scoring for the purpose of IVD calcification screening. Comparatively, MRI is the most effective and sensitive method for detecting pathologic biochemical changes in tissues, thereby allowing differentiation between different stages of the degenerative process in an IVD (Levitski et al., 1999; Sharp and Wheeler, 2005). Normal discs are easily identified due to the high water content of the NP, which provides high MR-signal intensity on T2-weighted images. Degenerated discs have reduced T2 signal intensity (Modic et al., 1988; Karkkainen et al., 1993), reflecting loss of water and proteoglycan content and decreased chondroitin-keratan sulfate ratios in the nucleus (Tertti et al., 1991). Mineralized chondrodystrophic dog discs also show decreased signal intensity owing to the calcified nucleus (Sether et al., 1990; Sharp and Wheeler, 2005). Low-field (0.2–0.23 T) MRI has been successfully used to grade IVD degeneration in dogs (Seiler et al., 2003; Bergknut et al., 2011a, 2011b). The Pfirrmann system (Pfirrmann et al., 2001) for grading lumbar

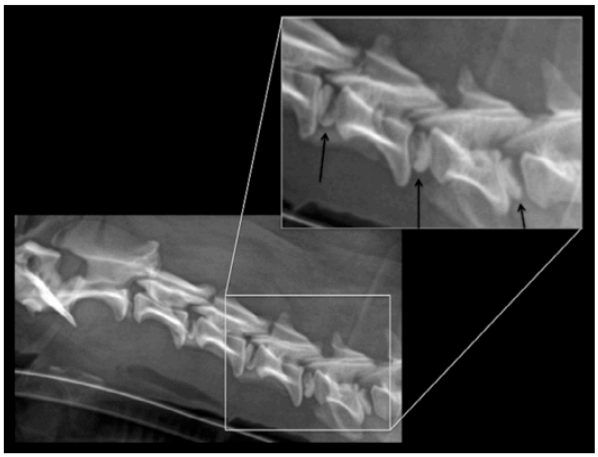

Fig. 2. Right-lateral radiograph of the cervical spine (C1-C7) of a Dachshund obtained for scoring of intervertebral disc calcification. Note several severely mineralized but non-displaced calcified intervertebral discs that are clearly visible radiographically. Inset, black arrows indicate radiographically detectable intervertebral disc calcification (RDIDC) at C4–5, C5–6 and C6–7.

IVD degeneration using MRI in people can be reliably used to grade IVD degeneration in all locations of the vertebral column in dogs of various breeds and ages (Bergknut et al., 2011a), and has substantial agreement with scores for the gross pathology-based Thompson system (Bergknut et al., 2011b).

Unfortunately CT and MRI have some disadvantages as screening tools, including limited availability and variable quality for veterinary usage, and increased cost of performing the studies (Robertson and Thrall, 2011). Additionally, MRI studies of the entire vertebral column may be time prohibitive and require general anesthesia to prevent patient motion (Robertson and Thrall, 2011). However, we suggest that the time to complete a non-contrast-enhanced CT study of the spine using a modern helical scanner would be no more than that to obtain precisely positioned spinal radiographs, and could be reasonably achieved using sedation. At this stage, the intra- and interobserver variation for scoring spinal radiographs for RDIDC has not been reported, although it is acknowledged that variation may exist and should be minimized (Stigen and Kolbjornsen, 2007; Jensen et al., 2008). The effectiveness of a screening program depends on high test accuracy, and therefore, the need exists for identification and quantification of the utility of alternate imaging modalities, and of potential variation between scorers.

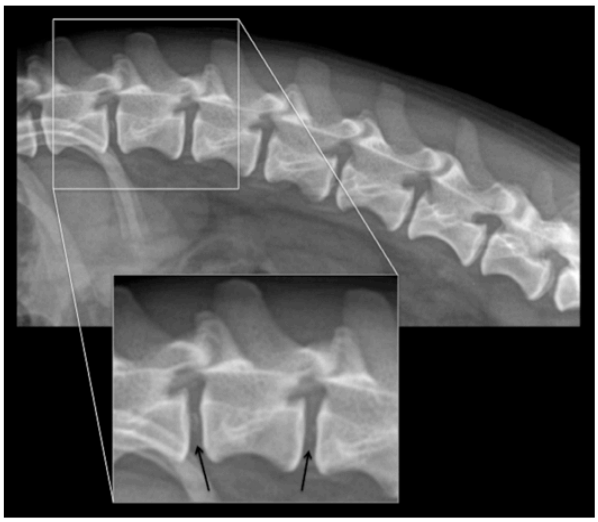

Fig. 3. Right-lateral radiograph of the lumbar spine (T13-S1) of a Dachshund obtained for scoring of intervertebral disc calcification. Inset, L1–2 and L2–3 intervertebral discs are only minimally calcified in situ (black arrows); compare to severely calcified discs in Fig. 2.

Effect of various physical factors on the occurrence of radiographically detectable intervertebral disc calcification and intervertebral disc herniation

Lappalainen et al. (2001) compared the relationship between curvature of the radius and ulna with the degree of RDIDC in 124 Finnish miniature Dachshunds to examine the hypothesis of Hansen (1964) that curvature of the legs is proportional to the degree of chondrodystrophy, and that by breeding straight-legged Dachshunds, the occurrence of IVDD could be reduced. No statistically significant difference was found in the mean radius/ulna curvature between groups of dogs that were healthy and without RDIDC, healthy with RDIDC, and diseased dogs with RDIDC; thus, radiographs of the radius and ulna are of no benefit in selecting dogs for breeding programs (Lappalainen et al., 2001).

Abnormal discs in chondrodystrophic dogs may be more sensitive to mechanical stress, and environmental factors imposing different loadings on discs may have either beneficial effects or accelerate the degeneration process (Jensen and Ersboll, 2000b). A study of 48 Dachshunds was conducted to evaluate the occurrence of RDIDC in light of numerous body conformation and exercise pattern variables (Jensen and Ersboll, 2000b). A beneficial relationship between moderate amounts of daily exercise and reduced RDIDC was demonstrated; however, vigorous, monotonous types of exercise (such as running next to a bicycle) tended toward an association with higher numbers of calcified discs. Additionally, this study found no reason to avoid moderate daily stair climbing as this seemed to reduce the rate of occurrence of RDIDC, and the effects of moderate duration of exercise and stair climbing appeared to be additive.

Perhaps surprisingly, the risk of RDIDC and acute thoracolumbar IVD herniation tended to increase with decreasing spinal length and tuber calcaneus-to-patella tendon distance (Jensen and Ersboll, 2000b; Levine et al., 2006), although longer dogs experienced more severe clinical signs if they were affected with IVD herniation (Levine et al., 2006). The findings of these two studies seem to contradict arguments that breeding toward a shortened type of Dachshund would decrease the severity of IVDD. Conversely, Packer et al. (2013) did find a significant positive association between an increased back length to height at the withers ratio and increased risk of thoracolumbar IVD extrusion, thus recommending that selection for extremely long backs should be discouraged. Hence, there is contradictory information regarding whether shorter or longer conformation might decrease the frequency and severity of IVDD. Being skeletally smaller or overweight is also associated with increased risk of thoracolumbar IVD extrusion (Packer et al., 2013).

Advances in genetic testing for radiographically detectable intervertebral disc calcification

Ball et al. (1982) recognized that the Dachshund was predisposed to IVDD, and concluded that certain lineages showed a hereditary predisposition for the disease (with a prevalence of up to 62% in some families), although not via a simple pattern of genetic transmission. These authors supported the idea of selective breeding to reduce the prevalence of IVDD (Ball et al., 1982). However, this was difficult to implement at the time because methods to detect

at-risk animals were not well established and affected animals are generally only revealed as middle-aged adults (i.e. 4–6 years of age).

Recently, a major susceptibility locus on chromosome 12 harboring genetic variants associated with the development of RDIDC in Dachshunds has been identified and validated in a genome-wide-association study of Danish Dachshunds (Mogensen et al., 2011, 2012). In these studies, dogs with ≥6 RDIDC (or dogs having undergone surgery for IVD herniation) were classified as cases, and dogs with 0–1 RDIDC were used as controls, to ensure a distinct phenotypic classification. The findings suggest a recessive-like pattern of inheritance for RDIDC, and support the idea that a limited number of loci underlie RDIDC expression in the Dachshund, but that the disease is most likely affected by additional genetic and environmental factors which are yet to be determined (Mogensen et al., 2011). Perhaps future studies on this RDIDC-associated region of the genome will result in the development of a DNA-based genetic test that can be utilized in breeding programs.

Conclusions

IVDD presents a significant health problem for the Dachshund breed, affecting many dogs and resulting in a potentially devastating outcome. As we describe here, there are a number of published studies supporting the correlation between IVD calcification and clinical IVDD in the Dachshund breed, as well as a hereditary basis for the severity of IVD degeneration, and therefore the number of disc calcifications, seen in an individual dog. A radiographic scoring scheme has been adopted by a number of Dachshund clubs and breeders around the world with the aim of reducing the incidence of IVDD through selective breeding. The effectiveness of this scheme has not yet been analyzed.

Test variability in scoring radiographs for the presence of RDIDC has not been evaluated, so future studies would be beneficial to establish that the test is accurate (both precise and true), and to determine which veterinary professionals might be best suited to performing the scoring. Additionally, investigation of alternative screening tools using imaging modalities other than radiography (e.g. CT or MRI) may provide a better method of identifying at-risk animals, both for breeding purposes and for use in conjunction with genetic testing. Further, longitudinal studies documenting the reduction in RDIDC and subsequently IVD herniations achieved through selective breeding using such scoring tools are also needed.

Conflict of interest statement

The authors of this paper do not have any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Chapter 2: Experiment 1 Publication

Precision of spinal radiographs as a screening test for intervertebral disc calcification in Dachshunds

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Certification:	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution

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Precision of spinal radiographs as a screening test for intervertebral disc calcification in Dachshunds

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ABSTRACT

Among dog breeds, the Dachshund has the highest lifetime incidence of intervertebral disc disease (IVDD). Intervertebral disc (IVD) calcification is an indicator of severe degeneration that predisposes to disc herniation. IVDD is heritable in Dachshunds, and in some countries, breeding candidates are screened to reduce IVDD occurrence by selecting dogs according to their score of radiographically detectable intervertebral disc calcification (RDIDC) and excluding dogs with ≥5 RDIDCs from breeding. This study evaluated the precision of scoring spinal radiographs for IVD calcification and subsequent classification of Dachshund dogs for breeding based on their RDIDC score. Digital radiographs of the spine were obtained in 19 clinically healthy, young adult Dachshunds, and scored for RDIDC independently by five scorers with varying levels of experience, three times each. Within scorer (repeatability) and between scorer (reproducibility) variability was estimated both at the individual IVD level and at the whole dog level for breeding classification purposes.

At the IVD level, some degree of scorer effect was supported by the pairwise repeatability (92.3%; 95% CI: 88.8–94.7%) being marginally higher than the reproducibility (89.2%; 95% CI: 85.7–91.8%). Scorer-specific patterns confirmed the presence of scorer subjectivity. Repeatability significantly increased with scorer experience but the reproducibility did not. RDIDC scoring repeatability and reproducibility substantially decreased at the cervicothoracic spine region, likely due to anatomical superimpositions. At the dog level, a breeding classification could be repeated by the same scorer for 83.6% (95% CI: 73.8–90.2%) of the dogs, and was reproduced between two scorers for 80.2% (95% CI: 66.6–89.1%) of the dogs. The repeatability of breeding classification also seemed to increase with scorer experience but not the reproducibility. Overall, RDIDC scoring revealed some degree of inconsistency explained by scorer subjectivity and inexperience, and anatomical superimpositions. Scorer training and experience is strongly recommended to improve test precision and ensure appropriate classification of Dachshunds for breeding.

1. Introduction

Intervertebral disc disease (IVDD) causes substantial morbidity and mortality in the Dachshund breed of dog, owing to the particularly high lifetime incidence risk of 15–25% (compared to 2–3.5% for the general dog population) (Goggin et al., 1970; Priester, 1976; Ball et al., 1982; Simpson, 1992; Bergknut et al., 2012). The Dachshund is a dog breed classified as having chondrodystrophy (i.e., disproportionate dwarfism; also reported as achondroplasia or hypochondroplasia) (Verheijen and Bouw, 1982; Simpson, 1992; Jensen and Christensen, 2000). In chondrodystrophic dogs, the intervertebral disc (IVD) undergoes chondroid metaplasia that is characterized by rapid maturation in which newly formed cells tend to be chondrocytes rather than fibrocytes (Hansen, 1952; Verheijen and Bouw, 1982; Simpson, 1992). By the time an affected Dachshund is 12–24 months of age, the majority of the formerly gelatinous nucleus pulposus of a disc may consist of hyaline carti-

Abbreviations: CT, computed tomography; GP, general practitioner; IVD, intervertebral disc; IVDD, intervertebral disc disease; PfA, perfect agreement; PwA, pairwise agreement; RDIDC, radiographically detectable intervertebral disc calcification.

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lage, which can subsequently calcify; this premature degenerative process occurs simultaneously but with varying severity in all discs along the vertebral column (Hansen, 1959; Ghosh et al., 1977; Bray and Burbidge, 1998). The now hardened nucleus pulposus is predisposed to herniate through the annulus fibrosus under minimal stress, resulting in explosive displacement of disc material into the vertebral canal (Hansen Type I disc disease) and compressive myelopathy (Hansen, 1952). The peak age of onset of clinical IVDD is between 4 and 6 years in the Dachshund (Gage, 1975; Priester, 1976; Aikawa et al., 2012b). Regrettably, 20–25% of chondrodystrophic dogs retain gait abnormalities and neurologic deficits following surgical treatment for IVDD (Scott, 1997; Olby et al., 2003; Aikawa et al., 2012a). Moreover, dogs with this condition may be euthanized for various owner-dependent reasons (Lappalainen et al., 2014).

More than 60 years ago, Hansen (1951) first postulated that there might be a relationship between the constitution of chondrodystrophic dogs, the development of their IVDs, and ensuing disc herniation (Hansen, 1951). Subsequently, several studies have investigated the relationship between radiographically detectable intervertebral disc calcification (RDIDC) – an indicator of severe disc degeneration – and the occurrence of clinical IVDD in Dachshunds, finding that the incidence of RDIDC increases with age and varies with size and hair coat type (Stigen, 1991, 1995, 1996; Lappalainen et al., 2001). Importantly, dogs with RDIDC are at substantially greater risk for developing signs of IVDD during their lifetime than those without RDIDC (Havranek-Balzaretti, 1980; Stigen, 1996; Lappalainen et al., 2001; Rohdin et al., 2010; Lappalainen et al., 2014). When specifically quantified, RDIDC score at 2 years of age was found to be a significant predictor of future IVD herniation (Jensen et al., 2008). Additionally, recurrence of IVD herniation, usually at a different location from the initial site, is more frequent in dogs with radiographic evidence of disc calcification at the time of first surgery (Mayhew et al., 2004; Rohdin et al., 2010; Brisson et al., 2011). It has also been demonstrated that dogs that have parents with RDIDC are more likely to have RDIDC themselves, supporting that RDIDC is heritable (Havranek-Balzaretti, 1980; Jensen and Christensen, 2000). Recent genome-wide-association studies have resulted in the discovery of a major susceptibility locus on chromosome 12 that harbors genetic variants associated with the development of RDIDC in Dachshunds, further supporting that RDIDC is hereditary (Mogensen et al., 2011, 2012). Given the high heritability for RDIDC in Dachshunds, selective breeding against disc calcification is recommended to reduce the occurrence of IVDD in this breed (Jensen and Christensen, 2000; Lappalainen et al., 2014).

The optimum age for conducting spinal radiographs to screen Dachshunds for RDIDC is 24–48 months, although dogs as young as 12 months and older than 48 months are scored (Jensen and Arnbjerg, 2001; Lappalainen et al., 2014). Several countries have initiated radiographic screening programs for RDIDC in young adult Dachshunds (Rohdin et al., 2010; Mogensen et al., 2011; Lappalainen et al., 2014). RDIDC is scored as the total count of calcified discs in the vertebral column between C2 and S1 (i.e., a discrete numerical score between 0 and 26) as identified on spinal radiographs, and recommendations to breed with dogs that have $\leq$2 RDIDCs, exclude dogs with $\geq$5 calcifications from breeding, and to use dogs with a score of 3–4 judiciously, are supported by research findings (Jensen and Christensen, 2000; Jensen et al., 2008; Mogensen et al., 2011; Lappalainen et al., 2014).

To accurately screen a dog for IVD calcification as part of a selective breeding program, a precise (i.e., consistent) test is necessary. Spinal radiography, and RDIDC scoring, is the most pragmatic, cost-effective and, often, the only accessible method to conduct in vivo screening of Dachshunds in practice. However, some degree of subjectivity is to be expected when interpreting radiographs, and an appreciation of the precision of RDIDC scoring and its influencing factors is lacking (Rosenblatt et al., 2014). Therefore, the aim of this study was to evaluate the precision of RDIDC scoring within and between scorers (i.e., repeatability and reproducibility, respectively). Variation in precision was investigated according to biological factors (IVD location within the spine) and scorer factors (degree of training and experience).

2. Materials and methods

2.1. Study subjects

Participating dogs were recruited from local Dachshund breeders and clinic caseload via word-of-mouth between April 1 and July 31, 2011, at the Companion Animal Health Centre (Roseworthy Campus, The University of Adelaide) using the following inclusion criteria: purebred Dachshund dog (confirmed with ANKC (Australian National Kennel Council) certificate of registration and pedigree, and corresponding microchip and/or tattoo), aged between 18 and 60 months (i.e., young adult), clinically healthy, and without current or prior manifestation of IVDD or other illness. The University of Adelaide Animal Ethics Committee granted approval for this study (project no.: S-2011-001), and informed owner consent was obtained for each dog included.

2.2. Radiography

Spinal radiographs were obtained for each dog, including at least five radiographs per study with the X-ray beam centered on and collimated for the cervical, cervicothoracic, thoracic, thoracolumbar, and lumbar spine regions (Jensen and Arnbjerg, 2001; Sharp and Wheeler, 2005; Jensen et al., 2008), to enhance assessment of each IVD space by minimizing artifacts associated with divergence of the X-ray beam (e.g., penumbra, parallax error). Radiographed dogs were under general anesthesia (using a protocol deemed appropriate for each patient by the administering board-certified anesthesiologist) and positioned in right lateral recumbency to facilitate repeatable positioning (Sharp and Wheeler, 2005). A digital radiographic system (Sedecal Reference DX; Madrid, Spain) was used to obtain the radiographs.

2.3. Scoring

The order of the radiographic studies was changed before they were distributed to five veterinarians (scorers), who reviewed them independently. The scorers were placed into one of three categories based on their previous experience with interpreting spine radiographs. The 'expert' category included one veterinary radiologist (co-author AKL) with over 10 years experience scoring for RDIDC. The 'specialist' category included two board-certified veterinary radiologists (co-authors SED and NSW) who had not previously scored for RDIDC in particular but routinely evaluate canine spinal radiographs. The 'general practitioner' (GP) category included two primary care veterinarians who had both been working in the profession for at least 7 years but did not have specific training in interpreting spinal radiographs (i.e., beyond standard tertiary veterinary education and day-to-day experience). Radiographic images were stored in a commercially available PACS (picture archiving and communication system) (iQ-View; IMAGE Information Systems Ltd., London, UK), which was used by some scorers for viewing images and making measurements. Other scorers remote from the institution used OsiriX Imaging Software (Pixmeo, Geneva, Switzerland). All scorers viewed the images in digital imaging and communications in medicine (DICOM) format using high-resolution, commercial-grade monitors, with freedom to manipulate (post-process) the images as they wished. Each

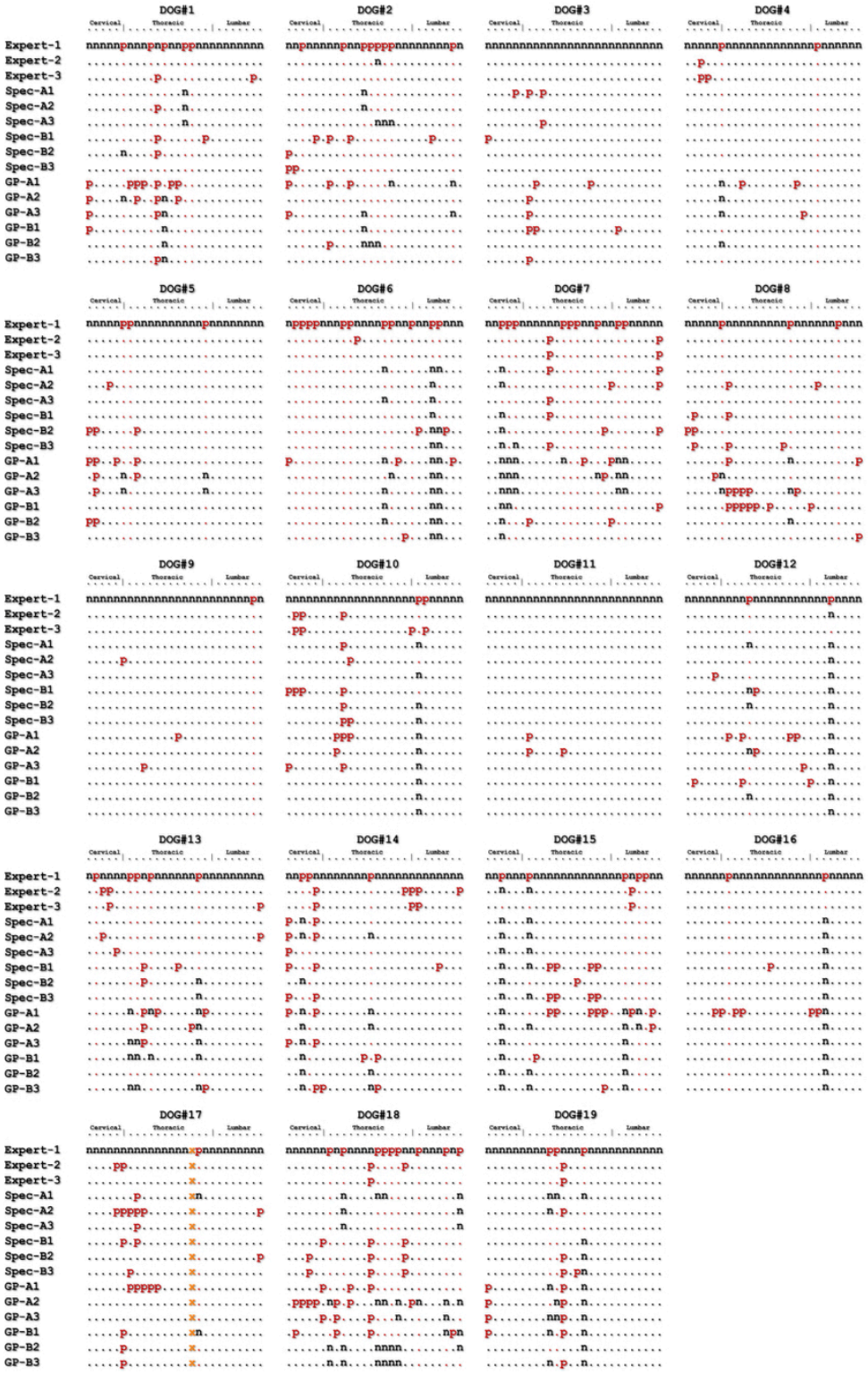

Fig. 1. RDIDC scoring—alignment of individual intervertebral disc (in column) scores of each scorer (expert, specialist A, specialist B, GP A, and GP B) and each iteration (1–3) (in row). Intervertebral discs (IVDs) are ordered according to their location within the vertebral column of each of the 19 participating Dachshund dogs. A "n" codes a negative score, a "p" codes for a positive score, a "dot" codes for a score agreeing with the first row score (iteration 1 of the expert), and "x" codes for an absent IVD due to fused vertebrae. "Spec": specialist, "GP": general practitioner.

radiographic examination was evaluated three times by all scorers, sequentially in the order in which they were provided, with a minimum one-month interval between rounds of scoring during which time they did not have access to the studies. The individual case reference number randomly assigned by the PACS was not masked on the images or changed between iterations; however, all other identifying patient information was removed. The scorers were instructed to identify and record all intervertebral discs between C2 and S1 that they believed were calcified using a custom scoring template. An IVD was recognized as calcified based on the subjective assessment of mineral opacity being present within an intervertebral disc space in the expected region of the nucleus pulposus. No time limit was imposed for assessing each study, however, scorers were not permitted to return to a study once they had completed their evaluation of it, or to review any prior studies.

2.4. Statistical analysis

Data were entered and collated using Microsoft Excel 2011 (Microsoft Corporation, Redmond, WA, USA) and all statistical analyses were conducted using Stata version 13.1 (Stata-Corp, College Station, TX, USA). Data and analysis codes are available for consultation upon request to the corresponding author.

The precision of RDIDC scoring was evaluated by estimating the repeatability and the reproducibility at the individual intervertebral disc level and at the whole dog level. Repeatability expresses the scoring consistency across the three replicates from a same scorer (agreement within scorer), while the reproducibility expresses the scoring consistency across the different scorers (agreement between scorers) (Dohoo et al., 2009a). Two types of agreement were estimated within and between scorers: 'perfect' agreement, which refers to the proportion (%) of RDIDC scores that were identical across the 3 replicates within a scorer or across the 15 replicates across the 5 scorers, and 'pairwise' agreement, which refers to the proportion (%) of RDIDC scores that were identical between a pair of replicates within a scorer or between a pair of replicates between scorers. In addition to conventional agreement estimation, agreement was also assessed using phylograms or distance trees built from the alignment of RDIDC scores and dog breeding classifications, as described by Caraguel et al. (2009) and using the phylogenetic package MEGA version 6 (Tamura et al., 2013). This approach visually represents the proximity between scoring iterations within and between scorers, and helps in identify scoring patterns. Cohen's kappa values were intentionally not used due to the well documented limitations of this agreement parameter (Byrt et al., 1993).

2.4.1. IVD classification agreement

Estimates of agreement and their confidence intervals were obtained by conducting separate logistic regression models to predict the probability of IVD classifications agreeing. Four separate datasets were reshaped into a long format to analyze separately the perfect and the pairwise repeatabilities and reproducibilities. To account for the fact that score comparisons were clustered within IVDs, and that IVDs were clustered within a dog, random effects for dogs and for IVDs within a dog were added to the model. Given that a same dog, and its IVDs, was scored by all scorers and each scorer scored all 19 dogs, scorer was added as a random effect cross-classified with dog and IVD. When modeling pairwise reproducibility, models including all random effects could not converge; therefore, pairwise reproducibility was investigated with models only using dog as a random effect.

Changes in agreement across individual IVD locations within the vertebral column (position 1 [C2-3] to 26 [L7-S1]), across the 3 major spine regions (cervical, positions 1–6; thoracic, positions 7–19; lumbar, positions 20–26), and across the scorers and their experience level (expert, specialist, GP), were compared by building separate unconditional models and including these factors as fixed effects. Interactions between scorer and biological factors were not investigated. Given the scale of the study, factors such as Dachshund type, age, or sex were not investigated here. When scorers or IVDs were included as a fixed effect into the model, the corresponding random effect was removed. When modeling pairwise reproducibility, all scorers and their experience level could not be modeled at once (each score comparison involved two scorers at once) and, therefore, each scorer and experience level was modeled as separate fixed effects in separate models.

Direct interpretation of the models' fixed parameters (intercepts and/or effect coefficient), ignoring random effects parameters, provided a cluster-specific interpretation of the agreement. To obtain mean agreement (i.e., population-averaged interpretation), the following approximation formula was used to convert cluster-specific to population-averaged predicted proportions and confidence interval limits (Dohoo et al., 2009b):

$$\text{Prob (positive score)} \approx \text{logit}^{-1}\left(\frac{(\beta_0 + \beta_X \text{Category\_X})}{\sqrt{\left(1 + 0.346 \times \left(\sigma^2_{\text{scorer}} + \sigma^2_{\text{dog}} + \sigma^2_{\text{IVD}}\right)\right)}}\right) \quad (1)$$

where β_0 is the model intercept coefficient; β_X Category_X is the category fixed effect (according to the model, either an individual IVD, spine region, scorer, or scorer experience level); σ^2_{scorer}, σ^2_{dog} and σ^2_{IVD} are the scorer, dog and IVD within dog random effect variance, respectively; and logit^{-1} is the inverse of the logit function ($\text{logit}^{-1}(x) = 1/(1+\exp^{(-x)})$). Post-regression inferences were two-sided and adjusted using the Bonferroni method (*alpha*, set at 5%, divided by the number of pairwise comparisons within the term) to account for the large number of pairwise comparisons across categories (26 IVDs, 3 spine regions, 5 scorers, and 3 scorer experience levels; e.g., up to 325 pairwise comparisons among the 26 IVDs). For pairwise reproducibility, post-regression inferences across scorers and experience levels were not feasible (separate models) and estimated differences were simply assessed using 95% confidence interval (95% CI).

2.4.2. Dog breeding classification agreement

Adapting the grading recommendations from radiographic screening programs in Finland, Denmark and Norway (Lappalainen et al., 2014), we binarized the classification of dog breeding status such that dogs with ≥5 RDIDCs identified by the scorer were classified at 'high risk' of transmitting IVDD if bred (i.e., positive at the dog level), and dogs with <5 RDIDCs identified by the scorer were classified at 'low risk' of transmitting IVDD if bred (i.e., negative at the dog level). Similar to individual IVD scoring, estimates of dog classification agreements and their confidence intervals were obtained by conducting separate logistic regression models to predict the probability of dog classifications agreeing. Cross-classified random effects between scorer and dog were included into the models (IVD level not present at the dog level classification). Changes in agreement across the scorers and their experience level were compared by building separate models and including these factors as fixed effects. When scorers were included as a fixed effect into the model, the corresponding random effect was not included in the model. Post-regression inferences were adjusted using the Bonferroni method. Models' fixed parameters were interpreted as population-averaged predicted proportions using Eq. (1). As for the IVD level, pairwise reproducibility could not be modeled with all scorers at once and, therefore, each scorer and experience level were modeled as separate fixed effects in separate models. Differences in pairwise agreement across scorers and their experience levels were assessed using 95% CI.

Fig. 2. RDIDC scoring—alignment of dog (in column) breeding classification for each scorer (expert, specialist A, specialist B, GP A and GP B) and iteration (1–3) (in row). A total of 19 Dachshund dogs participated in the study. A "n" codes a dog classified as "low risk" (i.e. <5 RDIDC; negative at the dog level), a "p" codes for a dog classified as 'high risk' dog (i.e., ≥5 RDIDC; positive at the dog level), and a "dot" codes for a dog classification agreeing with the first row (iteration 1 of the expert). "Spec": specialist, "GP": general practitioner.

3. Results

The study subjects were 19 client-owned Dachshund dogs of the following size and hair coat variants: standard smooth-haired ($n = 5$), miniature smooth-haired (8), miniature long-haired (5), and miniature wire-haired (1). In this group there were 5 intact males, 13 intact females, and 1 neutered female. The median age of the dogs was 36 months (range, 21–60 months).

In total, 493 intervertebral discs were examined (26 IVDs per dog, with one IVD in one dog excluded due to fused vertebrae) by each of the five scorers, three times separately (7395 scores recorded in total) (Fig. 1). At the dog level, the 19 dogs were classified as at 'high risk' for breeding (≥5 RDIDC) or at 'low risk' for breeding (<5 RDIDC) according to the findings of each of the five scorers, three times independently (Fig. 2).

3.1. Intervertebral disc level precision

Estimates and confidence intervals (95% CI) of 'perfect' agreement and 'pairwise' agreement of RDIDC scores within each scorer (repeatability) and across scorers (reproducibility) are summarized (Table 1, Fig. 3).

Overall, the agreement within scorer was higher than across scorers, and the perfect agreement (more stringent of the two assessments) was lower than the pairwise agreement. Across scorers, GP A had the lowest agreement with themselves and with the other scorers (significantly lower repeatability and reproducibility), while the expert was the most repeatable. Repeatability significantly increased with scorer experience but reproducibility did not. Regardless of perfect or pairwise estimates, IVD T1-2 (position 7) had the lowest repeatability and reproducibility, while IVDs L2-3 (position 21) and L5-6 (position 24) had the highest repeatability and reproducibility, respectively (Table 1, Fig. 3). Across the three spine regions, the lumbar region had the highest agreement within and between scorers compared to the cervical and thoracic regions.

Fig. 4 represents a phylogram (distance tree) of all scorer iterations for RDIDC scoring, where the length of the branches between two iterations is scaled based on the difference in RDIDC score between those two iterations. By inspection, the scoring iterations from a same scorer tended to be clustered (i.e., on the same branch or closely located to it), which indicates that most scorers had a consistent but distinct scoring pattern. Even though the branch distance between iterations within and between scorers was not very different (i.e., overall repeatability and reproducibility values approximate), the nature of the scoring pattern was distinct enough to separate scorers. GP A showed the lengthiest branches amongst their three iterations (lowest repeatability) and was the farthest from other scorer iterations (lowest reproducibility), while GP B showed similar length branches between their iterations and compared to specialist B (similar repeatability), and was closer to the specialists and expert with each subsequent iteration (i.e., improving reproducibility).

3.2. Dog level precision

Similar to agreement at the IVD level, across the 15 dog classification iterations, the agreement within scorer was higher than across scorers, and the perfect agreement was lower than the pairwise agreement (Table 2). Again, repeatability increased with experience but the reproducibility stayed similar across scorers and experience levels. GP A had the lowest repeatability and reproducibility. The pairwise repeatability of GP A was smaller than the pairwise reproducibility, revealing highly variable dog classification from this scorer. The expert had the highest repeatability and reproducibility.

No clustering or classification pattern was apparent within and between scorers' iterations in the dog classification phylogram (Fig. 5). This could be explained by the fact that only 19 dogs were studied and there was not enough resolution (power) to identify distinct classification patterns. In other words, the repeatability and reproducibility variability of dog classification overlapped.

4. Discussion

A highly precise and robust test is expected to have both repeatability and reproducibility that are very high and similar (i.e., no additional variability due to the scorer). In the case of RDIDC scoring, the pairwise repeatability (92.3%; 95% CI: 88.8–94.7%) was marginally higher than the pairwise reproducibility (89.2%; 95% CI: 85.7–91.8%), which revealed some degree of scorer-dependence in the scoring. Although the within and between scorer agreements appear high, the repeatability translates into 12.5% (0.923^{26}) of screened dogs that would have identical scores for all 26 IVDs when scored twice by the same scorer (i.e., 87.8% of dogs would have at least one IVD score that could not be repeated by a scorer); and the reproducibility translates into 5.1% (0.892^{26}) of screened dogs that would have identical scores for all 26 IVDs if scored by two different scorers (i.e., 94.9% of dogs would have at least one IVD score that could not be reproduced between two scorers) (this assumes the worst case scenario where there is complete independence of individual IVD scoring).

The scoring iterations from a same scorer showed consistent but distinct scoring patterns, with scorer-specific clusters of branches in the phylogram (Fig. 4). This finding supports that scoring for RDIDC is somewhat subjective and depends on the individual

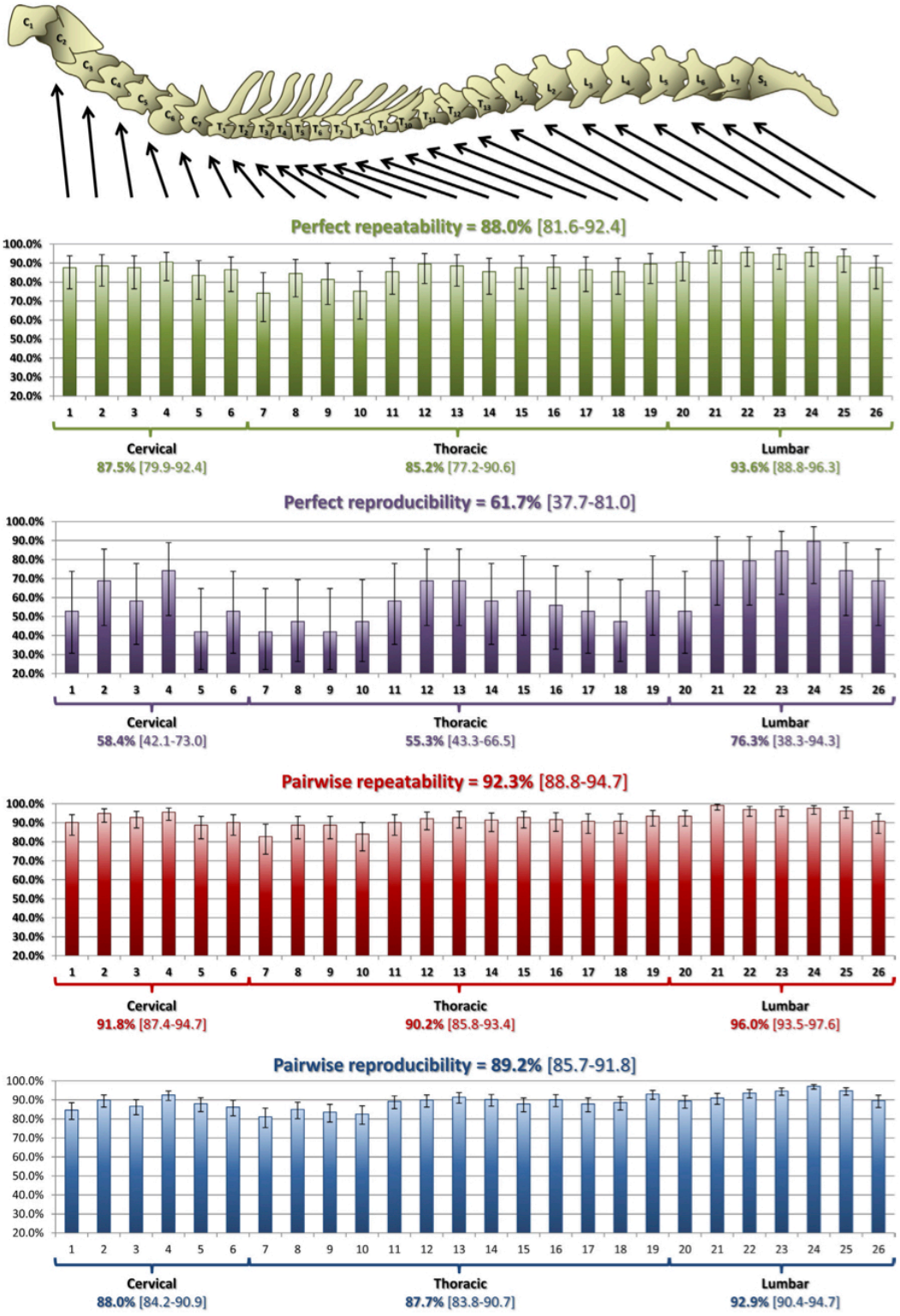

Fig. 3. Modeled estimates of perfect, and pairwise, repeatability and reproducibility of RDIDC scores, for each of the 26 intervertebral discs scored, for the cervical, thoracic and lumbar regions of the spine, and overall. The 95% confidence intervals are reported in brackets or using 'error' bars and do not include any Bonferroni adjustment. "C1–C7": cervical vertebrae, "T1–T13": thoracic vertebrae, "L1–L7": lumbar vertebrae, "S1": first sacral vertebra.

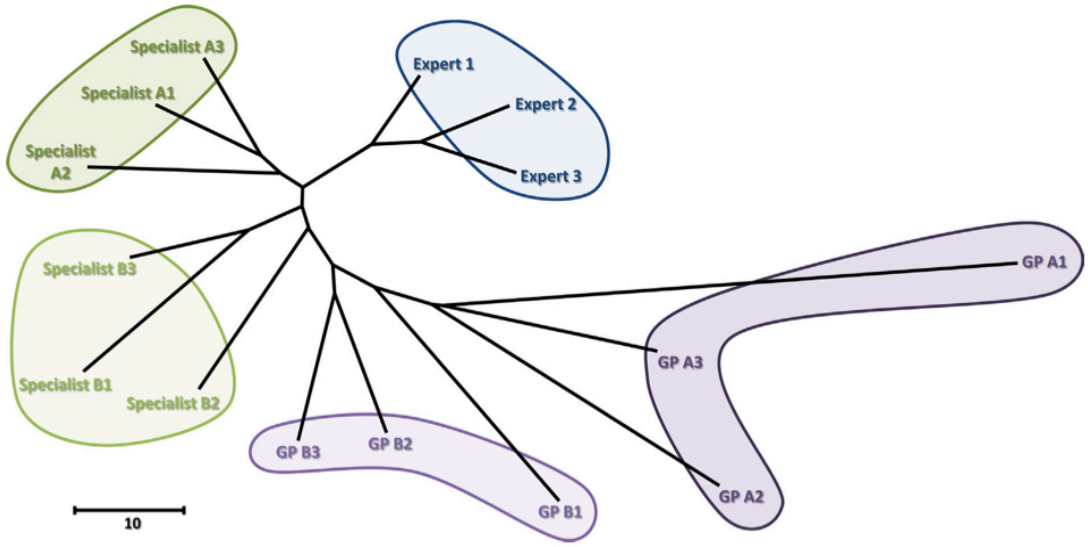

Fig. 4. Phylogram representing the agreement amongst scorers when scoring for RDIDC. One expert, two specialists (A and B), and two general practitioners (GP A and B) assessed each intervertebral disc from the 19 participating Dachshund dogs three times independently (iterations 1–3). The length of the branches between two scorer iterations represents their relative disagreement and is scaled based on the number of differing RDIDC scores out of the 493 intervertebral discs assessed by each scorer (scale bar = 10 differences).

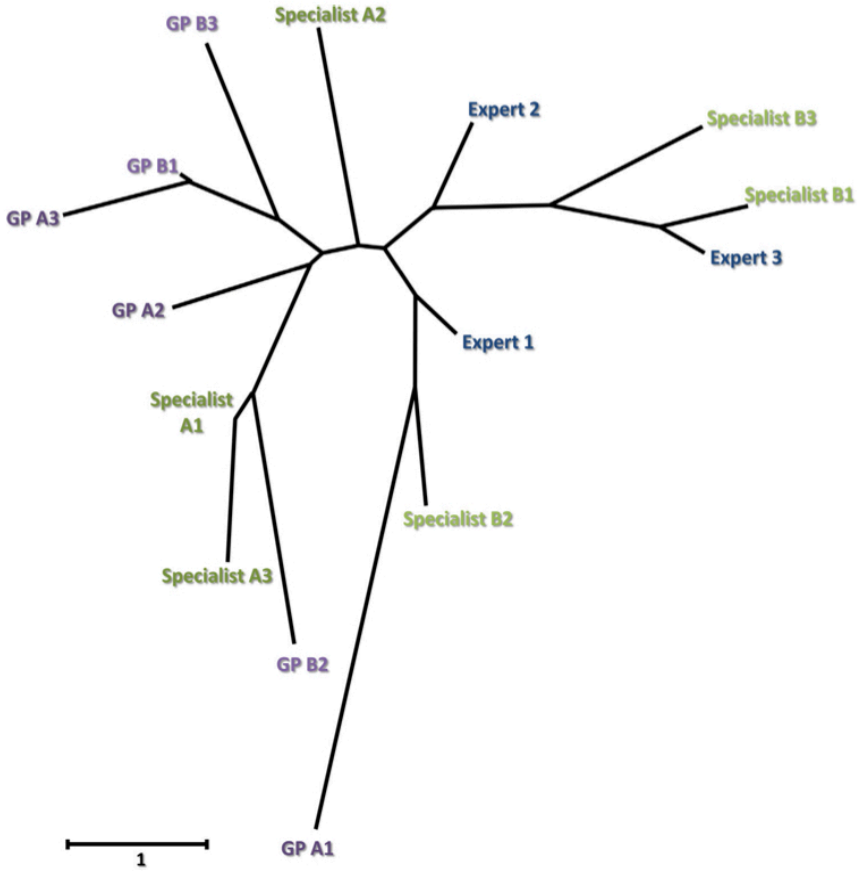

Fig. 5. Phylogram representing the agreement amongst scorers when classifying 19 Dachshund dogs for breeding based on RDIDC score. One expert, two specialists (A and B), and two general practitioners (GP A and B) assessed each dog three times independently (iterations 1–3). The length of the branches between two scorer iterations represents their relative disagreement and is scaled based on the number of differing classifications out of the 19 possible classifications (scale bar = 1 difference).

Table 1
Perfect and pairwise agreement (95% confidence interval) of radiographically detectable intervertebral disc calcification (RDIDC) scores within and between scorers, across the scorers and their experience level, and across the individual intervertebral discs (IVD) and the spine regions (cervical, thoracic, lumbar). Perfect agreement refers to the proportion (%) of RDIDC scores which were identical across the 3 replicates within a scorer or across the 15 replicates across the 5 scorers. Pairwise agreement refers to the proportion (%) of RDIDC scores which were identical between a pair of replicates within a scorer or between a pair of replicates between scorers. "GP": general practitioner. Within a predicting factor (scorer, experience level, IVD position, spine region), the categories sharing a same superscripted letter are not significantly different at the 5% level (after Bonferroni adjustment for multiple comparisons).

	Perfect agreement (95% CI)		Pairwise agreement (95% CI)	
	Within scorer (%)	Across scorers (%)	Within scorer (%)	Across scorers (%)[a]
Scorers				
Expert	93.7[a] (90.6–95.7)	–	95.1[a] (93.2–96.4)	89.6[a] (86.3–92.2)
Specialist A	91.6[ab] (88.1–94.2)	–	93.8[ab] (91.5–95.4)	90.6[a] (90.2–91.0)
Specialist B	89.4[ab] (85.3–92.4)	–	92.4[b] (89.8–94.3)	90.1[a] (89.7–90.5)
GP A	75.9 (69.8–81.1)	–	86.5 (82.6–89.6)	86.2 (81.9–89.5)
GP B	88.1[b] (83.7–91.3)	–	91.6[b] (88.8–93.7)	89.7[a] (86.3–92.2)
Experience level				
Expert	93.7[a] (88.5–96.6)	–	95.3[a] (92.8–96.9)	89.6[a] (86.3–92.2)
Specialist	90.5[ab] (85.5–93.9)	–	93.4[ab] (90.7–95.3)	90.0[a] (86.7–92.4)
GP	82.6[b] (75.0–88.2)	–	89.7[b] (85.8–92.5)	88.0[a] (84.2–90.9)
Intervertebral disc				
1	87.5[a] (76.4–93.8)	52.7[a] (30.7–73.7)	90.1[a] (83.4–94.2)	84.7[abcd] (79.7–88.5)
2	88.5[a] (77.8–94.4)	68.8[a] (45.2–85.4)	94.8[a] (90.2–97.3)	89.9[fghijk] (86.2–92.6)
3	87.5[a] (76.4–93.8)	58.1[a] (35.3–77.9)	92.8[a] (87.2–96.0)	86.7[bcdefg] (82.2–90.1)
4	90.5[a] (80.7–95.6)	74.0[a] (50.5–88.8)	95.5[a] (91.2–97.7)	92.6[ijkl] (89.7–94.6)
5	83.5[a] (70.8–91.2)	41.9[a] (22.1–64.7)	88.8[a] (81.5–93.3)	88.0[cdefgh] (83.8–91.1)
6	86.5[a] (75.0–93.1)	52.7[a] (30.7–73.7)	90.1[a] (83.4–94.2)	86.2[bcdef] (81.6–89.7)
7	74.1[a] (59.2–84.9)	41.9[a] (22.1–64.7)	82.7[a] (73.4–89.2)	81.1[a] (75.4–85.6)
8	84.5[a] (72.2–91.9)	47.3[a] (26.3–69.3)	88.8[a] (81.5–93.3)	85.0[abcde] (80.1–88.8)
9	81.4[a] (68.1–89.9)	41.9[a] (22.1–64.7)	88.8[a] (81.5–93.3)	83.5[abc] (78.3-87.6)
10	75.2[a] (60.4–85.7)	47.3[a] (26.3–69.3)	84.0[a] (75.1–90.1)	82.5[ab] (77.1-86.8)
11	85.5[a] (73.6-92.5)	58.1[a] (35.3–77.9)	90.1[a] (83.4–94.2)	89.2[defghij] (85.4–92.1)
12	89.5[a] (79.2–95.0)	68.8[a] (45.2–85.4)	92.1[a] (86.2–95.5)	89.9[fghijk] (86.2–92.6)
13	88.5[a] (77.8–94.4)	68.8[a] (45.2–85.4)	92.8[a] (87.2–96.0)	91.5[hijkl] (88.2–93.8)
14	85.5[a] (73.6–92.5)	58.1[a] (35.3–77.9)	91.4[a] (85.3–95.1)	90.2[fghijk] (86.6–92.8)
15	87.5[a] (76.4–93.8)	63.4[a] (40.1–81.8)	92.8[a] (87.2–96.0)	87.9[cdefgh] (83.7–91.0)
16	87.8[a] (76.5–94.0)	55.8[a] (32.8–76.6)	91.6[a] (85.4–95.2)	90.1[fghijk] (86.4–92.7)
17	86.5[a] (75.0–93.1)	52.7[a] (30.7–73.7)	90.8[a] (84.3–94.7)	87.9[cdefgh] (83.7–91.0)
18	85.5[a] (73.6–92.5)	47.3[a] (26.3–69.3)	90.8[a] (84.3–94.7)	88.7[defghi] (84.7–91.6)
19	89.5[a] (79.2–95.0)	63.4[a] (40.1–81.8)	93.5[a] (88.2–96.4)	93.0[jkl] (90.3–95.0)
20	90.5[a] (80.7–95.6)	52.7[a] (30.7–73.7)	93.5[a] (88.2–96.4)	89.4[efghi]j (85.6–92.2)
21	96.6[a] (89.8–98.9)	79.3[a] (56.0–92.0)	99.1[a] (96.7–99.7)	91.0[ghijkl] (87.6–93.4)
22	95.6[a] (88.2–98.4)	79.3[a] (56.0–92.0)	96.9[a] (93.4–98.5)	93.6[kl] (91.0–95.4)
23	94.5[a] (86.7–97.8)	84.4[a] (61.6–94.8)	96.9[a] (93.4–98.5)	94.6[lm] (92.3–96.2)
24	95.6[a] (88.2–98.4)	89.5[a] (67.3–97.2)	97.6[a] (94.4–98.9)	97.1[m] (95.6–98.0)
25	93.5[a] (85.1–97.3)	74.0[a] (50.5–88.8)	96.2[a] (92.3–98.1)	94.8[lm] (92.5–96.3)
26	87.5[a] (76.4–93.8)	68.8[a] (45.2–85.4)	90.8[a] (84.3–94.7)	89.7[efghijk] (85.9–92.4)
Spine region				
Cervical	87.5[a] (79.9–92.4)	58.4[a] (42.1–73.0)	91.8[ab] (87.4–94.7)	88.0[a] (84.2–90.9)
Thoracic	85.2[a] (77.2–90.6)	55.3[a] (43.3–66.5)	90.2[a] (85.8–93.4)	87.7[a] (83.8–90.7)
Lumbar	93.6 (88.8–96.3)	76.3[a] (38.3–94.3)	96.0[b] (93.5–97.6)	92.9 (90.4–94.7)
Overall	88.0 (81.6–92.4)	61.7 (37.7–81.0)	92.3 (88.8–94.7)	89.2 (85.7–91.8)

[a] Significance between categories within scorers and experience level was assessed using 95% CI.

scorer. Repeatability and reproducibility increased with the experience of the scorer. Also, self-training of a scorer appeared to be beneficial to improve the overall precision. Both GPs showed an apparent improvement in agreement after their first scoring iteration. This may reflect a gain in experience of these two scorers over the duration of the study. A larger scale study involving a greater number of inexperienced scorers, conducted over a longer period of time, would be necessary to refine this preliminary observation.

Regardless of the scorer, the cervicothoracic region of the spine (i.e., C6-T2; positions 5-7) seemed to be particularly difficult to score compared to other regions (Fig. 3). This difficulty is likely due to superimposition of anatomy, namely the scapulae and/or rib heads. Despite the inherent limitation of the anatomy, RDIDC can reach a high level of precision with increased experience as illustrated by the expert's repeatability.

RDIDC scoring is most meaningful at the dog level when screening potential breeding Dachshunds. Albeit slightly higher, the probability to repeat (same scorer) the breeding classification of a dog approximated the probability to reproduce (two different scorers) this classification (83.6% and 80.2%, respectively). Therefore, there was no evidence that scorer subjectivity substantially impacted the precision of the dog classification. Still, the repeatability of dog classification increased with the experience of the scorer, thereby confirming that such experience and/or training are beneficial to consistently classify dogs for breeding (i.e., greater precision). However, further evaluation is needed to confirm that experience also improves the accuracy of dog classification.

Overall, RDIDC scoring provides enough room for some scorers to be subjective and directed guidance and training are recommended to improve the precision of the test, particularly with inexperienced scorers.

Our study population included 19 young adult Dachshund dogs without current or prior history of clinical IVDD, most of which were candidates for breeding in South Australia. We believe that the participating dogs were a fair representation of the spectrum of Dachshund candidates for RDIDC scoring in Australia. There was

Table 2
Perfect and pairwise agreement (95% confidence interval) of dog classification for breeding within and between scorers, and across the scorers and their experience level. Perfect agreement refers to the proportion (%) of dog classifications which were identical across the 3 replicates within a scorer or across the 15 replicates across the 5 scorers. Pairwise agreement refers to the proportion (%) of dog classifications which were identical between a pair of replicates within a scorer or between a pair of replicates between scorers. "GP": general practitioner. Within a predicting factor (scorer or experience level), the categories sharing a same superscripted letter are not significantly different at the 5% level (after Bonferroni adjustment for multiple comparisons).

	Perfect agreement (95% CI)		Pairwise agreement (95% CI)	
	Within scorer (%)	Between scorers (%)	Within scorer (%)	Between scorers (%)[a]
Scorers				
Expert	84.5[a] (60.4–95.0)	–	89.4[a] (77.5–95.4)	81.8[a] (68.7–90.2)
Specialist A	79.3[a] (55.0–92.2)	–	86.2[a] (73.2–93.4)	80.8[a] (67.3–89.6)
Specialist B	79.3[a] (55.0–92.2)	–	86.2[a] (73.2–93.4)	79.4[a] (65.4–88.8)
GP A	58.1[a] (35.2–77.9)	–	72.8[a] (57.2–84.3)	78.8[a] (64.6–88.4)
GP B	74.1[a] (49.8–89.1)	–	82.9[a] (69.0–91.4)	80.0[a] (66.1–89.1)
Experience level				
Expert	84.4[a] (60.4–95.0)	–	89.4[a] (77.5–95.4)	81.8[a] (68.7–90.2)
Specialist	79.2[a] (62.0–89.9)	–	86.2[a] (76.1–92.4)	80.4[a] (66.8–89.3)
GP	66.1[a] (48.6–80.1)	–	77.9[a] (65.8–86.6)	79.5[a] (65.6–88.7)
Overall	75.0 (63.1–84.0)	31.6 (8.6–54.6)	83.6 (73.8–90.2)	80.2 (66.6–89.1)

[a] Significance between categories was assessed using 95% CI.

no substantial difference in these dogs' demographics (e.g., age, size, hair coat variant, RDIDC score; see Section 3 for detail) from previous studies (Havranek-Balzaretti, 1980; Stigen, 1991, 1995, 1996; Lappalainen et al., 2001; Rohdin et al., 2010), and therefore, the extrapolation of this study's estimates to other Dachshund populations may be acceptable. The number of dogs participating was relatively small ($n = 19$) but an increase in the number of dogs would have marginally helped in discriminating dog-level estimates which where numerically very close. At the IVD level, the study included a total of 493 IVDs scored 15 times each, which resulted into a total of 7395 within scorer pairwise comparisons and 44,370 between scorer comparisons, leaving little concern about the power of the study.

On the digital radiographic studies, each participating case was assigned a unique reference number during the anonymization process; this number could not be masked or changed on the images between iterations for the same scorer. However, the scorers were blinded to all other recognizable demographic information. Given that a one-month or greater interval occurred between scoring iterations, and scorers were not allowed to return to a case once its evaluation was completed, the impact of a potential review bias should be minimal (Ransohoff and Feinstein, 1978).

To mimic the actual variability that occurs when veterinarians interpret and score radiographs, we chose not to completely standardize the viewing conditions under which the radiographic images were scored. The quality of the spinal radiographs obtained for screening purposes will also influence the precision of the test. All radiographs for this study were obtained under repeatable, controlled conditions in a referral veterinary institution with high quality digital X-ray equipment and trained personnel, and viewed by the scorers under similar, high-quality conditions. Such facilities are not available in all veterinary practices and, typically, radiographs are obtained from various clinics and sent to a remote scorer for interpretation; therefore, broad variation in radiographic quality is expected. Moreover, the potential difference in test precision when using screen-film radiography compared to digital radiography was not investigated, but this variable is expected to influence results. Hence, the reported precision of RDIDC scoring herein might be higher than could be expected in all situations.

Consistent with the literature (Bille et al., 2012), the process of obtaining a series of lateral–lateral spinal radiographs in systemically healthy, young adult, anesthetized dogs was safe, with all participating dogs recovering uneventfully from the procedure, and no complications noted or reported by owners. However, all anesthetic procedures carry some risk, and the chance of disc herniation or other back injury occurring whilst a dog is anesthetized cannot be completely eliminated. The potential benefit, both to the individual dog and breeder, and to the breed as a whole, would seem to outweigh this minimal risk.

A relatively new initiative by the Dansk Gravhundeklub (2013) (Danish Dachshund Club) is the calculation of breeding values for individual dogs from the available information of all animals in a given pedigree, and only dogs with a breeding value above the breed average are recommended for use (Mogensen et al., 2011, 2012). Regardless of the method used for determining suitable dogs for breeding, there has been an obvious need for longitudinal studies in Dachshunds that document the successful reduction in RDIDC and clinical IVDD, achieved through selective breeding using this radiographic screening tool. A recent paper has shown that RDIDC (especially scores ≥5) and the development of clinical IVDD are strongly associated with one another, and that spinal radiography is an appropriate tool for screening breeding dogs to select against RDIDC and IVDD (Lappalainen et al., 2014).

An alternative screening test with even greater precision may be considered, especially for the cervicothoracic spine region as a small peak in both RDIDC and histopathologically-identified disc calcification is reported here (Hansen, 1952; Stigen, 1996; Jensen and Arnbjerg, 2001; Rohdin et al., 2010). Compared to radiography, computed tomography (CT) eliminates difficulties with superimposition because it is a cross-sectional modality, and also provides superior contrast resolution and image-reformatting capabilities (Hecht et al., 2009). We speculate that CT represents the future of IVD calcification screening in Dachshunds, although cost and availability may be limiting factors.

5. Conclusions

When utilized as a screening test for IVD calcification in Dachshunds, RDIDC scoring revealed some scorer subjectivity regardless of scorer experience. However, the test's precision improved substantially with increased scorer experience. Therefore, it is recommended that scoring RDIDC be performed by veterinarians who have particular experience interpreting spinal radiographs (e.g., specialty training in veterinary radiology). Additionally, spinal radiography has inherent limitations including anatomic superimpositions at the cervicothoracic spine region, and the investigation of an alternative screening test utilizing a cross-sectional imaging modality such as CT is warranted.

Conflict of interest

The authors of this paper do not have any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Chapter 3: Experiment 2 Manuscript (unpublished)

Scorer and modality agreement for the detection of intervertebral disc calcification in Dachshunds

Statement of Authorship

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SCORER AND MODALITY AGREEMENT FOR THE DETECTION OF INTERVERTEBRAL DISC CALCIFICATION IN DACHSHUNDS

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Key words: Dachshund, intervertebral disc calcification, scoring, radiography, CT, MRI

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Abstract

The Dachshund is a chondrodystrophic breed of dog predisposed to premature degeneration and calcification, and subsequent herniation, of intervertebral discs (IVDs). This condition is heritable in Dachshunds and breeding candidates are screened for radiographically detectable intervertebral disc calcification (RDIDC), a prognostic factor for clinical disease. RDIDC has been previously shown to be consistent within scorers, however, strong scorer effect (subjectivity) was also reported. The aim of this study was to estimate the within- and between-scorer agreement (repeatability and reproducibility, respectively) of computed tomography (CT) and magnetic resonance imaging (MRI) for scoring IVD calcification and to compare these modalities with radiography.

Twenty-one Dachshund dogs were screened for IVD calcification using the three imaging modalities. Each case was scored twice independently by three scorers. Repeatability was highest for radiography (95.4%), and significantly higher than for CT (90.4%) but not MRI (93.8%). Reproducibility was also highest for radiography (92.9%), but not significantly higher than CT or MRI (89.4% and 86.4%, respectively). Despite good agreement, previous evidence of scorer subjectivity with radiography was confirmed, which was not generally observed with CT and MRI. Overall, CT scored IVDs differently than radiography and MRI (64.8% and 62.7% agreement, respectively), while radiography and MRI scored more similarly (85.7% agreement). The increased consistency of radiography may be related to prior scorer experience with the modality and RDIDC scoring. This study does not support replacing radiography with CT or MRI to screen for heritable IVD calcification in breeding Dachshunds.

Keywords: repeatability and reproducibility, Dachshund, radiography, CT, MRI, intervertebral disc calcification

Abbreviations:

IVD – intervertebral disc

IVDD – intervertebral disc disease

RDIDC – radiographically detectable intervertebral disc calcification

CT – computed tomography

MRI – magnetic resonance imaging

Introduction

Of all the dog breeds, the Dachshund has the highest lifetime incidence of intervertebral disc disease (IVDD)[1,2]. The results of a recent UK study, based on a survey of Dachshund owners ("Dachs-Life 2015"), found an overall IVDD prevalence of 15.7% in the surveyed Dachshund population of 1,975 dogs, with a significant prevalence range between different breed variants (7.1%-24.4%)[3]. This high prevalence may be due to a variety of genetic, physical and lifestyle-related factors[3], but is likely primarily attributable to their chondrodystrophic morphology. Dogs with chondrodystrophy undergo chrondroid metaplasia, the premature maturation and degeneration of intervertebral discs (IVDs) that often results in calcification, an indicator of severe degeneration[2,4,5]. These degenerated IVDs are predisposed to herniate (displace) into the spinal canal under minimal stress, resulting in spinal cord compression and injury[4]. Dachshunds with IVD herniation have a high level of morbidity and mortality, and despite treatment that often includes complex and costly surgical intervention, a substantial proportion of dogs retain neurologic deficits[6-8]. IVDD is widely accepted as the Dachshund breed's greatest health problem.

Radiographically detectable intervertebral disc calcification (RDIDC) is highly heritable in Dachshunds[9-13], and the development of RDIDC at a young adult age corresponds with an increased risk of developing clinical IVDD during the lifetime of the dog[8,9,14-17]. Therefore, screening young adult breeding candidates for RDIDC, ideally at 24-30 months of age, can reduce the prevalence of the disease in the breed[10,17,18]. RDIDC is scored from a range of 0 to a maximum of 26 (i.e. 26 total IVDs in the canine cervical, thoracic and lumbar spine). Current screening programs recommend that Dachshunds with RDIDC scores of ≤ 2 are suitable for breeding, dogs with scores of 3-4 should be bred judiciously, and animals with scores ≥ 5 should be excluded for breeding purposes[8,10,11,16,17]. A comprehensive review of radiographic scoring for intervertebral disc calcification in the Dachshund is available[19] .

For a screening test to be useful in a selective breeding program, it must be precise. Recent evaluation of within- and between-scorer agreement for RDIDC scoring identified an overall high level of repeatability and reproducibility, but also identified some limitations of radiography as a screening tool[20]. Test precision was influenced by scorer experience level (expert scorer > specialist radiologist > general practitioner), which in turn affected the consistency (agreement) of the results. Individual scorer-dependent subjectivity was also identified.

The absence of RDIDC does not exclude a disc from being degenerative nor calcified, and only a portion of IVD calcifications present in a spine would be expected to be detected radiographically[16,21]. It is postulated that a cross-sectional imaging modality such as computed tomography (CT) would be a superior alternative for screening dogs for IVD calcification compared to radiography, as CT reduces challenges associated with anatomic superimposition and has improved contrast resolution[22,23]. Alternatively, magnetic resonance imaging (MRI) is a cross-sectional modality with superior contrast resolution to both CT and radiography, and high-field MRI is considered the optimal modality for imaging the spine[24,25]. MRI of intervertebral discs allows identification of earlier stages of disc degeneration than calcification due to its ability to detect biochemical changes in tissues, including loss of water and proteoglycan content and decreased chondroitin-keratan sulfate ratio in the nucleus pulposus, such that degenerative and calcified IVDs have decreased MR signal intensity[22,26-29]. IVD degeneration in the canine spine can be reliably graded using low-field MRI and the Pfirrmann classification system, which is based on lumbar IVD degeneration in people and has been verified with the gross pathology-based Thompson system[30-33].

The precision of CT and MRI scoring of IVD calcification in Dachshunds has not been assessed. Thus, the objectives of this study were to: (i) compare the precision of three diagnostic imaging modalities (radiography, CT and MRI) by estimating repeatability and reproducibility, (ii) estimate and compare the robustness (i.e. scorer independence) of each modality, and (iii) estimate the agreement across the three modalities for the detection of IVD calcification. It was anticipated that both CT and MRI would be more precise than radiography due to the cross-sectional nature of these modalities. However, it was expected that MRI would not completely agree with the two other modalities because this modality assesses various stages of IVD degeneration, not only calcification.

Methods

Study subjects

Dogs were prospectively recruited from Finnish Dachshund breeders through The Dachshund Club of Finland, between 22 November 2011 and 7 March 2012. Eligibility criteria included: purebred registered Standard Dachshund dog, young adult age (24 - 48 months old), and clinically healthy. Dogs were excluded if they had prior or current signs of intervertebral disc disease (IVDD) or other illness. Dogs were enrolled in the study with informed owner consent, and the study was approved and conducted with ethics approval from the National Animal Experiment Board of Finland (approval number, ESAVI/5794/04.10.03/2011).

Diagnostic imaging

The imaging was performed at the University of Helsinki Veterinary Teaching Hospital. Three diagnostic imaging modalities were employed to image the dogs' spines – radiography, computed tomography (CT) and low-field magnetic resonance imaging (MRI) (**Figure 1**). All imaging was performed within a single hospital visit, with the dogs under heavy sedation or general

anaesthesia. Radiography and CT were conducted on all dogs, while MRI was optional and based on owner preference.

Radiography

Spinal radiographs of the cervical, thoracic and lumbar regions were obtained for each dog using a previously described protocol[20] and a digital radiographic system (CPI Indico 100, Ontario, Canada). A minimum of five diagnostic quality radiographs was acquired for each dog.

Computed Tomography (CT)

CT was performed using a 2-slice helical scanner (Siemens Somatom Emotion Duo, Forchheim, Germany) with the following scanning parameters: 100 mA, 110 kV, 1.0 mm acquisition slice thickness, feed/rotation 2 mm, rotation time 0.8 s, reconstruction interval 0.5 mm, bone algorithm (WL, 500; WW, 3500). CT scanner limitations (i.e. excess tube heat) did not allow for scanning of the entire spine. The thoracolumbar spine was of greatest interest due to the propensity for clinical IVDD in this region. Therefore, T5-L7 (or a portion thereof) was scanned in all dogs. Where possible, the cervicothoracic (C6-T2) and/or the lumbosacral (L7-S1) spine junctions were also scanned; these regions were selected as they are anecdotally challenging to score radiographically for IVD calcification due to issues with superimposition of anatomy.

Magnetic Resonance Imaging (MRI)

MRI studies of the thoracolumbar spine were obtained using a low-field scanner (Vet-MR 0.23T, Esaote S.p.A, Genoa, Italy) and the following pulse sequences: sagittal plane T1W (TR, 510; TE, 18), sagittal plane T2W (TR, 2800; TE, 80), and transverse plane T1W (TR, 830; TE, 18). As with the CT imaging, the limitations of using a low-field magnet (specifically, acquisition time) did

not allow for imaging of the entire spine, so the thoracolumbar spine (T5-S1, or part thereof) was scanned, being the region of greatest clinical interest.

Scoring

Three veterinarians who all had diagnostic imaging backgrounds and training but varying levels of RDIDC scoring experience performed the scoring of the intervertebral discs. All cases were duplicated, coded (with individual identifying information removed from the images), and randomly ordered prior to distribution to ensure blinding of the scorers. The imaging studies were viewed in Digital Imaging and Communications in Medicine (DICOM) format using OsiriX image viewing software (Pixmeo, Geneva, Switzerland) and high resolution/brightness, commercial-grade monitors, with freedom to post-process images as preferred by the individual. The scorers recorded results for each imaging study using custom scoring templates, as per a previous study[20]. Scoring decisions were made by independent opinion. Observers were aware that the dogs were clinically healthy but were otherwise blinded to patient details and other identifiers.

Each radiographic study was scored for the presence or absence of IVD calcification. The CT cases were distributed one month after the radiographic scoring had been completed to facilitate scorer blinding. The presence or absence of IVD calcification was recorded, as was scorer confidence in the decision and approximate percentage of calcification of the total disc area (in 10% increments, 0-100%). Again, MRI cases were distributed one month after all scorers had completed the CT scoring. Based primarily on the sagittal T2W images[32], IVDs were graded for degeneration following the Pfirrmann classification scheme[30,33], which uses visual analysis of the IVD structure, distinction between nucleus pulposus and annulus fibrosis, MR signal intensity, and height of the

IVD to grade a disc on a scale of 1 (normal) to 5 (severe degeneration). Scorers were provided with example images and written description of the characteristics of each grade as a reference.

Statistical analysis

Scores were collected, collated and formatted using Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). An IVD score was classified as positive for calcification when calcification (≥ 10%) was observed (radiographs and CT) or when the Pfirrmann grade was ≥ 3 (MRI), and classified as negative otherwise. Analyses for study objectives (i) and (iii) were conducted using the statistical package Stata version 14.2 (Stata Corp, College Station, TX, USA), and analysis for objective (ii) was conducted using the phylogenetic package MEGA version 7[34]. Datasets and stata analysis codes are available upon request.

Modalities' repeatability and reproducibility

Precision was evaluated by estimating the repeatability and reproducibility of the three modalities. For a given modality, repeatability was estimated as the proportion of pairs of scores that agreed within a given scorer. The reproducibility was measured as the proportion of pairs of scores that agreed between two scorers. To compare precision across modalities, separate datasets and logistic models were developed for repeatability and reproducibility. The datasets were reformatted in a long format with each observation reporting an agreement (coded as "1") or a disagreement (coded as "0") between two scorer iterations for a given dog's IVD from a same scorer (repeatability dataset) or from two separate scorers (reproducibility dataset) of a given modality. Covariate factors included dog, IVD, modality, and scorer for each observation. Given that agreement observations were clustered within IVDs and IVDs were clustered within dogs, random effects for dog and IVD were added to the models to account for the lack of independence across observations. Also, given

that the study dogs and their IVDs were scored up to 6 times by a same scorer (clustered within scorers), scorer was included as a random effect cross-classified with dog and IVD. When modeling reproducibility, models with cross-classified structure could not converge and the reproducibility was modeled using scorers' pair, dog, and IVD random effect without cross-classification. Repeatability and reproducibility across modalities were estimated and compared by including modality as a fixed effect in the respective models.

The direct interpretation of the models' coefficients (intercepts and/or effect coefficient), ignoring random effects, provides cluster-specific estimates of agreement. To obtain average estimates across dogs, scorers and IVDs (i.e. population-averaged interpretation), cluster-specific predicted agreements and the limits of the 95% confidence interval were converted to population-averaged values using the following approximation formula[35]:

$$Prob(agreement) \approx logit^{-1}((\beta_0+\beta_1\ Modality)/\sqrt{(1+0.346*(\sigma^2_{scorer}+\sigma^2_{dog}+\sigma^2_{IVD}))}) \quad (1)$$

where β_0 is the model intercept coefficient; $\beta_1\ Modality$ is the modality fixed effect (radiography set as default category); σ^2_{scorer}, σ^2_{dog} and σ^2_{IVD} are the scorer, dog and IVD within dog random effect variance, respectively; and $logit^{-1}$ is the inverse of the logit function ($logit^{-1}(x) = 1/(1+e^{-x})$). Post-regression inferences were two-sided and adjusted using the Bonferroni method (alpha, set at 5%, divided by the number of pairwise comparisons between modalities, $alpha_{Bonferroni} = 1.7\%$).

Agreement across modalities

Agreement across modalities was estimated as the proportion of pairs of scores between modalities' iterations that agreed within a given scorer. Comparisons between scorer iterations were ignored to exclude between-scorer effect. The same data structure, model building, and population-

averaged interpretation as for repeatability and reproducibility were used. Agreement across modalities was explored across all Pfirrmann grade cut-offs (i.e. ≥ 1 to = 5).

Modalities' robustness (scorer independence)

The ruggedness of a test is defined as the capacity of a test to resist expected variation across users[36]. In other words, ruggedness measures how dependent the outcome of the test is on the person running or interpreting the test. Here, the ruggedness of each modality was investigated by determining the existence of scorer subjectivity when interpreting IVDs using a diagnostic imaging test. Similar to a previous report[20] and following the principle of a cluster analysis, distance-based Neighbor-Joining phylograms were built from an alignment of IVD scores (IVDs in columns and scoring iterations in rows) to identify the presence of iteration cluster(s) corresponding to distinct scoring pattern. If the two scoring iterations from a same scorer cluster together, there is evidence that the scoring from this scorer is distinct from the other scorers. To assess the robustness of the node linking two iterations together, bootstrap support values (proportion of resampled trees that include the node of interest) were generated using bootstrap-resampling 1,000 times and reported as a percentage on the nodes of the original tree[37]. A node with a bootstrap support value of ≥ 70% was considered robust. The advantage of this approach is that it accounts for both the quantitative distance and the qualitative pattern across scoring iterations.

Results

Study subjects

Twenty-one young adult (age range, 26-45 months; median, 30 months; SD, 4.8 months) Dachshund dogs were recruited. The study population was relatively homogeneous, with dogs being intact females (n = 10), intact males (9), neutered female (1) and neutered male (1), breed variants

being standard long-haired (11) or standard wire-haired (10), and weighing 7.6-12.6 kg (mean, 9.8 kg; SD, 1.3 kg).

Precision and robustness of each modality

A summary of the score for each available IVD in each dog, for each scorer, each iteration and each modality, is presented in **Figure 2**. Estimates and 95% confidence intervals (95% CI) of repeatability (within-scorer agreement) and reproducibility (between-scorer agreement) are reported (**Table 1**).

Radiography

Except for the C2-3 IVD of dogs #4 and #21 (**Figure 2**), all 26 potential IVDs from the 21 participating dogs (544 IVDs in total) were examined radiographically by each of the three scorers, two times independently (total, 3,264 scores). The repeatability of radiography was slightly higher than its reproducibility suggesting at first little scorer effect (**Table 1**). However, the phylogram (distance tree) of IVD scoring using radiography identified three clear clusters, corresponding to each individual scorer, supported by high bootstrap values (> 70%) (**Figure 3**). This revealed that each scorer had a scoring pattern that was unique enough to be discriminated from the other scorers. The length of the branches between two iterations reflects the amount of disagreement between these iterations (i.e. the shorter the branch length, the stronger the agreement between two iterations). Within each scorer, the distance between the iterations of scorer B were clearly longer than for scorers A and C, showing a lower repeatability for scorer B. Across scorers, scorer B was further away from the other two scorers corresponding to poorer reproducibility for this scorer.

Computed Tomography (CT)

Only a fraction of the IVDs, ranging from 8 to 19 per dog, were scanned using CT, providing a total of 314 IVDs scored. Overall, a total of 1,880 CT scores were obtained from the 6 scoring iterations, with four scores missing (**Figure 2**). The reproducibility of CT for scoring IVD calcification approximated its repeatability, which suggested no scorer effect (**Table 1**). Indeed, the CT phylogram (**Figure 4**) indicated no evidence of clear clusters (all bootstrap values < 70%), confirming a lack of evidence of scorer effect (subjectivity) with CT. The distances between iterations within a scorer and between scorers were similar but long, producing a starfish shaped tree. This reflects lower within-scorer agreement (repeatability) across all scorers compared to radiography, which subsequently resulted in lower between-scorer agreement (reproducibility).

Magnetic Resonance Imaging (MRI)

MRI scans were only available for 11 of the participating dogs and, at most, 14 IVDs per dog were examined. Overall, 142 IVDs were scored with a total of 840 MRI scores obtained from the 6 scoring iterations. The repeatability of MRI was moderately higher than its reproducibility (**Table 1**). The MRI phylogram (**Figure 5**) identified one strong cluster (bootstrap value 100%) corresponding to scorer B. This suggested that scorer B's interpretation of MR images was significantly different from the other two scorers (i.e. lower reproducibility for this scorer). The distance between the iterations within scorer B were also clearly longer compared to the iterations within each of the other two scorers, reflecting a lower repeatability for scorer B.

Comparison of modalities' repeatability and reproducibility

Across the three diagnostic imaging modalities, radiography showed the highest repeatability (95.4%) for scoring IVD calcification, and was significantly higher than CT (90.4%) but not significantly higher than MRI (93.8%) (**Table 1**). There was no significant difference in

reproducibility across the three modalities; however, a trend was present with decreasing between-scorer agreement for radiography, followed by CT and then MRI (92.9%, 89.4% and 86.4%, respectively).

Agreement between modalities

Regardless of the Pfirrmann grade cut-off used to binarize data into a 'positive' or 'negative' score for IVD calcification, CT moderately agreed with radiography (approximately 65% agreement) (**Table 2**). Agreement between MRI and the other two modalities substantially increased at the cut-off ≥ 3 and was the best at cut-off ≥ 4. However, agreements between modalities at the cut-offs between ≥ 3 and = 5 approximated. At cut-off ≥ 4, MRI and radiography agreed 85.4% of the time (95% CI, 80.3%-89.3%), while MRI and CT agreed 64.9% of the time (95% CI, 56.5%-72.4%).

Of all three modalities, considerably more IVD calcification was identified by CT (38.8% of all CT scores were positive for calcification) than radiography (8.2% of all radiography scores) and MRI (3.0% of all MRI scores).

Discussion

Due to the heritability of IVDD and IVD calcification in Dachshunds, selective breeding is important to reduce transmission to offspring[10,13,38]. Scoring IVDs for calcification is a reliable predictor of future IVDD development[17], and IVD calcification is currently screened for using conventional radiography. It was predicted that CT and MRI would provide better precision and less subjectivity than radiography when scoring for IVD calcification, as these cross-sectional imaging modalities reduce the confounding effects of anatomic superimposition and provide superior

contrast resolution[25]. Despite expectations, neither the repeatability nor reproducibility of CT or MRI was better than the repeatability and reproducibility of radiography. While the repeatability of MRI was similar to that of radiography, the repeatability of CT was significantly less. The reproducibility of both CT and MRI were less than that of radiography, however these were not significantly different. As anticipated for all modalities, estimates of repeatability were higher than estimates of reproducibility, although the two values were very similar for CT. The similar repeatability and reproducibility for CT indicates a lack of individual scorer subjectivity for this modality. Challenges with scoring IVD calcification using CT could have been due to less experience and/or training using this method of screening compared to radiography. Conversely, CT detected substantially greater overall numbers of calcified IVDs than the other modalities, including discs with smaller total proportion of calcification. This may have led to decreased scorer confidence in assigning a positive or negative score to a given IVD and thus greater variability between scoring iterations.

While the repeatability and reproducibility estimates were similar for both radiography and CT, MRI showed a larger discrepancy between repeatability and reproducibility. The lower level of reproducibility for MRI could be explained by the clear difference in scoring pattern of scorer B compared to scorers A and C (**Figure 5**). It is unclear which of the scorers were scoring most correctly (i.e. accurately); regardless, it could be concluded that a degree of difficulty arose when using MRI to screen for IVD calcification, possibly attributable to a lack of experience or training using MRI and the Pfirrmann grading system. On the other hand, our findings are similar to those of others who have evaluated the reliability of the Pfirrmann MRI classification system[30,33,39]. When the system was initially evaluated in people, the intra- and inter-observer agreement yielded average kappa scores of 0.88 and 0.77, respectively, with percentage agreements that approximated our

results (90.8% and 83.0%, respectively)[33]. A subsequent reliability study was conducted using a modified Pfirrmann grading system, and the intra- and inter-reader agreement remained good but comparatively less (Avg. K scores, 0.86 and 0.66, respectively; Avg. % agreement, 84.9% and 66.8%, respectively)[39]. Variable intra- and inter-observer agreement for scoring canine IVDs for degeneration using the Pfirrmann grading system has been identified (K score range, 0.58 to 0.93)[30,40]. We chose not to use conventional kappa values because of the recognised limitations of this method including its sensitivity to prevalence[41], which limits direct comparison between our agreement estimates and the kappa results obtained in other studies.

The Pfirrmann grading system is based on identifying progressive phases of IVD degeneration[30,33], not specifically IVD calcification. Although this means that our estimates of agreement for scoring IVD calcification between the different modalities cannot be considered equal, a cut-off Pfirrmann grade ≥ 3 was selected to assign a 'positive' score for IVD calcification on MRI. We chose this cut-off as grades of 3, 4 and 5 are assigned to IVDs with changes (reduced MR signal intensity and distinction between nucleus pulposus and annulus fibrosus) that would be expected with more severe IVD degeneration, potentially including some degree of calcification[32,42]. Further, it is recognised that discriminating between Pfirrmann grades 1 and 2, and between grades 3 and 4, can be challenging and subjective[30,33,39], supporting the choice to categorise scores of ≤ 2 as negative and ≥ 3 as positive for calcification. The agreement estimates between modalities at cut-off ≥ 3 approximated those at cut-offs ≥ 4 and = 5 (**Table 2**).

The recommendation that RDIDC scoring be performed by experts is further supported by the higher precision found in this study where the scorers had specific experience in diagnostic imaging, compared to our prior study using a heterogeneous group of scorers with variable

background[20]. Based on the agreement estimates identified herein, the chance of every IVD within a given dog being scored identically when evaluated twice by the same person (repeatability) is 29.4% (0.954^{26}), compared to 12.5% seen previously[20]. Similarly for reproducibility, when a given dog is scored twice by two different scorers the chance of every IVD within that dog being identically scored is 14.6% (0.929^{26}), compared to 5.1%. These calculations assume complete independence of individual IVD scoring, which is the worst-case scenario.

Radiography was the only modality of the three to show a clear scorer pattern (i.e. subjectivity), demonstrated as three distinct scoring clusters (**Figure 3**). These findings agree with those from our earlier work[20]. The scorer-dependent patterns demonstrated in that study were attributed to scorer differences that might be explained by variation in scoring ability and experience (general practitioner, specialist radiologist, and expert scorer). Comparatively, in the present experiment the scorers had a more similar background and training in diagnostic imaging; therefore, the observed subjectivity is less likely to be attributed to scorer experience but instead may be due to distinct individual scoring styles that could feasibly develop with greater experience. Nevertheless, of the three modalities evaluated radiography provides consistently higher within- and between-scorer agreement across all 26 potential IVDs, and when the highest level of precision in IVD calcification scoring is desired, radiography should be considered above CT and MRI.

The agreement estimates across the three modalities showed that MRI and radiography agreed more with each other than CT did with either modality. More agreement between radiography and CT might be initially expected as both modalities assess IVD calcification specifically, whereas MRI scoring is based on a wider spectrum of IVD degeneration. However, the lack of modality agreement between radiography and CT, and MRI and CT, is likely due to the

substantially larger number of IVD calcifications detected using CT versus the other modalities. The potential benefits of this higher detection rate using CT need further investigation. Although the relatively good agreement between radiography and low-field MRI (85.7%) could make MRI an acceptable alternative to RDIDC scoring when performed by an individual who is experienced using the Pfirrmann grading system, MRI is substantially more expensive and time consuming to perform than radiography, making it an impractical screening tool for dog breeders. However in experimental situations, use of a modified Pfirrmann grading system that is more discriminatory in determining severity of disc degeneration in Dachshunds, such as the one developed for elderly people[39], may be warranted.

The results of this study suggest that further insight into the accuracy of each modality is required before considering replacement of radiography with CT or MRI for IVD calcification screening in Dachshunds. As might be expected, the three modalities appeared to detect distinct features of IVD degeneration. While it seems that radiography is the best method of IVD screening in terms of precision, it is suspected that CT is in fact scoring more correctly—that is, CT is more accurate—than radiography and MRI, resulting in the disagreement of CT scores with radiography and MRI. If CT or MRI were shown to be more accurate than radiography, any gains achieved would need to be balanced with the increased cost, reduced access to the modality in veterinary practice, and overall feasibility for breeders.

Potential limitations of this study might be related to the CT and MRI equipment used, as whole dog spines could not be imaged because of technical limitations, thereby reducing the number of IVDs that were sampled and scored. However, the total number of scores obtained for each modality by the duplicate iterations for each of three scorers was sufficiently high for analysis.

Further, low-field MRI has known limitations in terms of image quality compared to high-field MRI; nevertheless, the literature indicates that low-field MRI is suitable for grading IVD degeneration in dogs[28,30-32]. The moderately inconsistent number and position of IVDs imaged by the various modalities in different dogs could have caused human counting errors when identifying which IVD was being scored at a given time; however, visual examination of the score summary diagram (**Figure 2**) did not identify patterns suggestive of frequent counting or localisation errors.

Conclusion

While it might be anticipated that more advanced screening modalities, namely CT and MRI, would improve diagnosis of IVD calcification compared to radiographic scoring, this study did not find any improvement in repeatability or reproducibility of those modalities. If an alternative modality were to replace radiography, training in modality-specific scoring should be implemented to increase within-and between-scorer agreement and test robustness. With correct scorer instruction, CT and MRI have the potential to increase the precision of IVD calcification screening. However, it is important to first evaluate the accuracy of CT and MRI to provide appropriate recommendations regarding which, if any, of the alternative modalities should replace radiography for the screening of IVD calcification in Dachshunds.

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Conflict of Interest Statement

The authors of this paper do not have any financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of this paper.

Tables

Modality	Within scorer agreement (Repeatability)	Between scorer agreement (Reproducibility)
Radiography	95.4%[b] (92.4-97.3)	92.9%[a] (67.8-98.8)
CT	90.4%[a] (84.8-94.1)	89.4%[a] (62.8-97.7)
MRI	93.8%[a,b] (88.9-96.6)	86.4%[a] (60.4-96.4)

Table 1. Model estimates of the repeatability and reproducibility for IVD calcification scoring by radiography, computed tomography (CT) and magnetic resonance imaging (MRI) (interpreted as positive if Pfirrmann grade ≥3), with 95% confidence intervals reported in brackets. Within a column, different superscript letters indicate significant differences between modalities.

Compared modalities	Pfirrmann Grade ≥ 1	Pfirrmann Grade ≥ 2	Pfirrmann Grade ≥ 3	Pfirrmann Grade ≥ 4	Pfirrmann Grade = 5
Radiography vs. CT	64.2% (58.5-69.4)	64.4% (58.5-69.9)	65.6% (58.0-72.5)	67.0% (59.0-74.2)	67.1% (58.9-74.3)
Radiography vs. MRI	20.1% (16.2-24.6)	46.4% (40.1-52.8)	80.8% (75.1-85.4)	85.4% (80.3-89.3)	83.9% (78.4-88.2)
CT vs. MRI	45.9% (39.9-52.0)	51.1% (44.6-57.5)	62.8% (54.8-70.0)	64.9% (56.5-72.4)	62.8% (54.2-70.7)

Table 2. Model estimates (95% CI) of pairwise agreement between scoring modalities for each Pfirrmann grade cut-off. CT: computed tomography; MRI: magnetic resonance imaging.

Figures

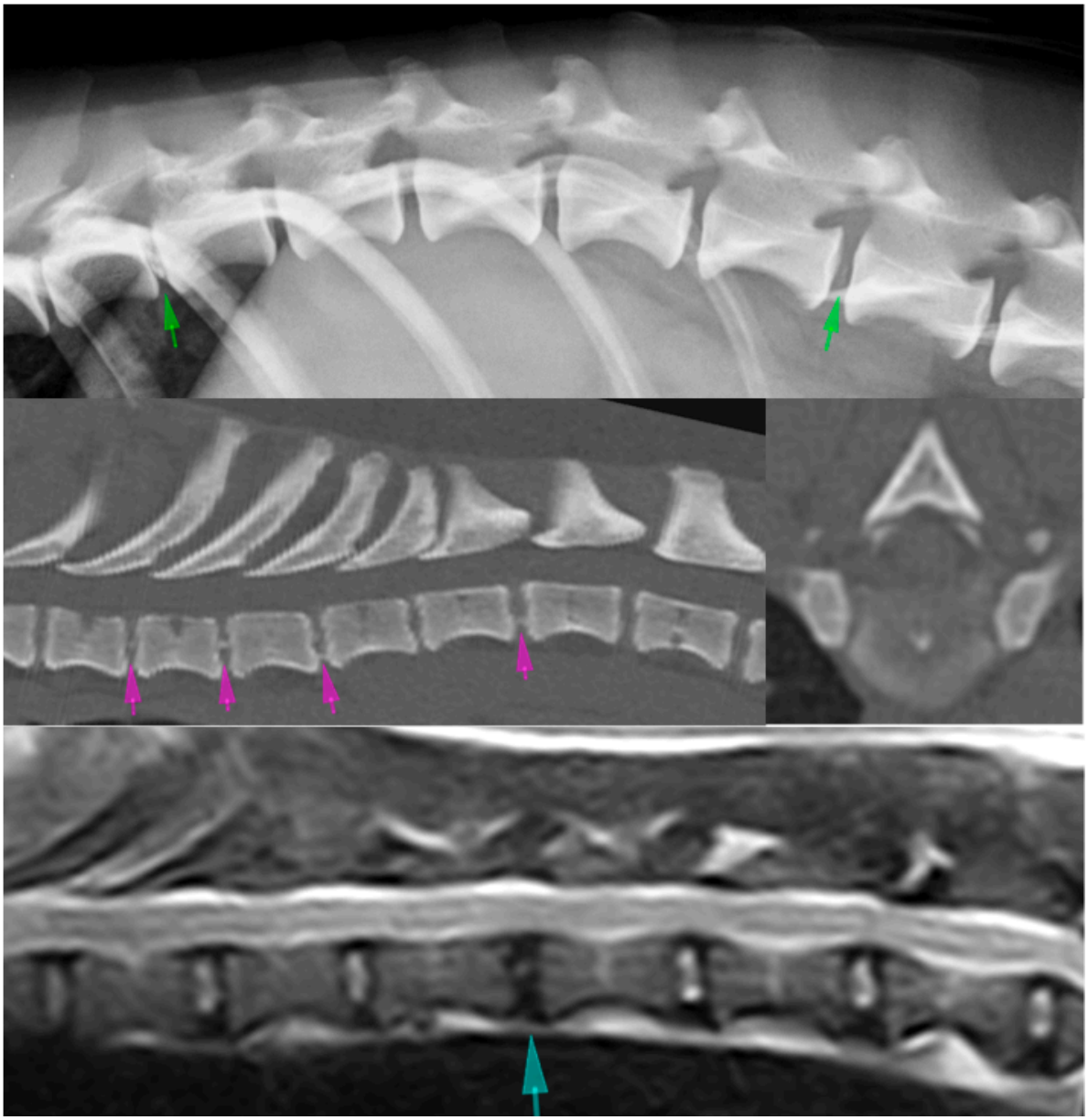
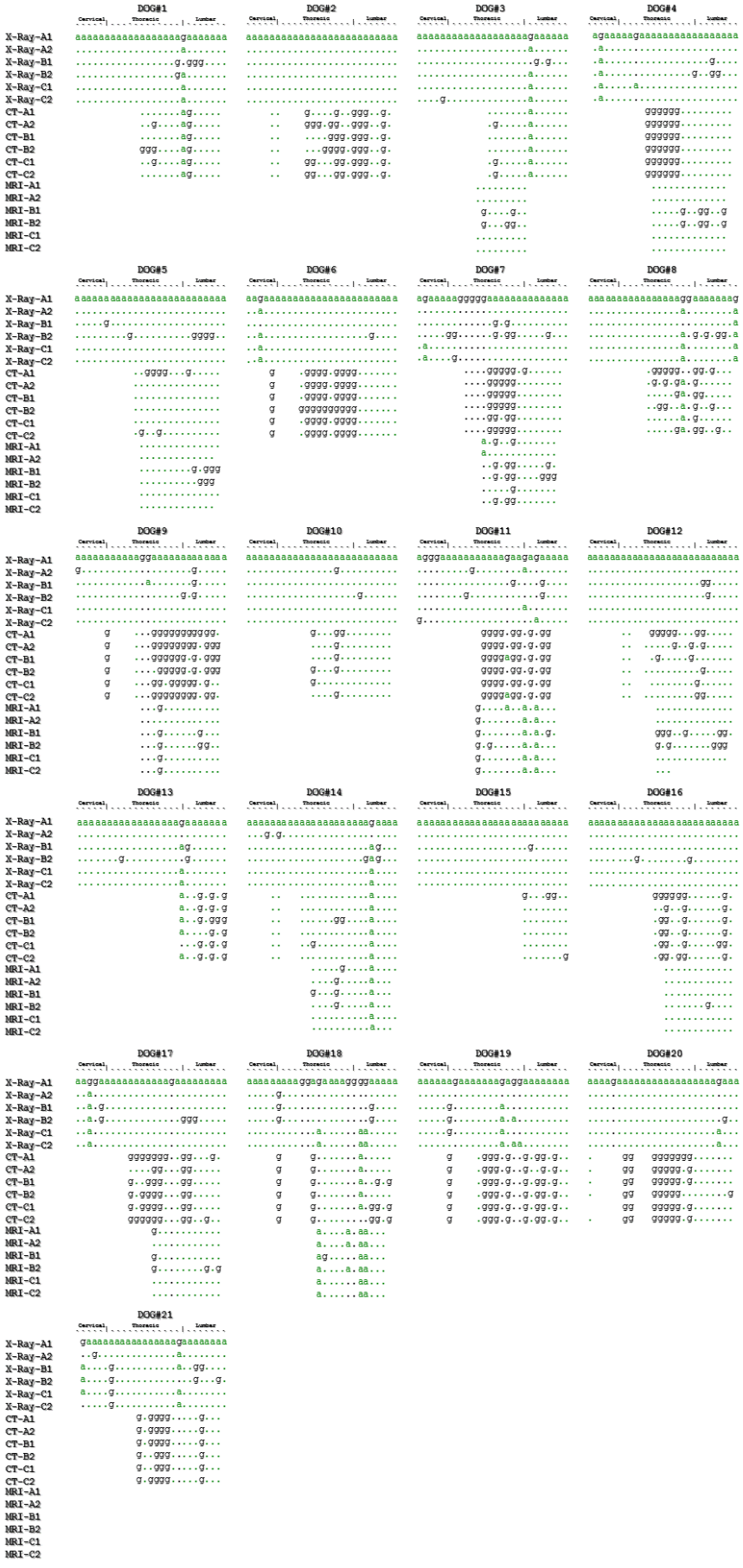


Figure 1. Example radiographic (top), CT (middle) and MRI (bottom) images obtained for IVD scoring (not necessarily from the same dog). The images are centered on the caudal thoracic spine. Example intervertebral disc calcifications are indicated on the radiograph (green arrows), and on the sagittal (pink arrows) and transverse plane CT images displayed in a bone window. On the T2W sagittal MR image, the blue arrow indicates a Pfirrmann grade 3 degenerative IVD. CT: computed tomography; MRI: magnetic resonance imaging.


DOG#1
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DOG#19
DOG#20
DOG#21
Cervical
Thoracic
Lumbar
X-Ray-A1
X-Ray-A2
X-Ray-B1
X-Ray-B2
X-Ray-C1
X-Ray-C2
CT-A1
CT-A2
CT-B1
CT-B2
CT-C1
CT-C2
MRI-A1
MRI-A2
MRI-B1
MRI-B2
MRI-C1
MRI-C2

Figure 2. Scoring alignment of individual intervertebral discs scored (column) by each scorer (A, B and C) for each iteration (1 and 2) and each modality (X-ray, CT and MRI) (row). The intervertebral discs (IVDs) of each of the 21 participating Dachshund dogs are ordered per their location in the vertebral column i.e. position 1 (C2-3) to 26 (L7-S1). An "a" codes for a negative score, a "g" codes for a positive score, a "dot" codes for a score that agrees with the first row (X-ray iteration 1 of scorer A), and a "blank" codes for an absent IVD score due to missing data. "X-ray" denotes radiography; "CT" denotes computed tomography; "MRI" denotes magnetic resonance imaging.

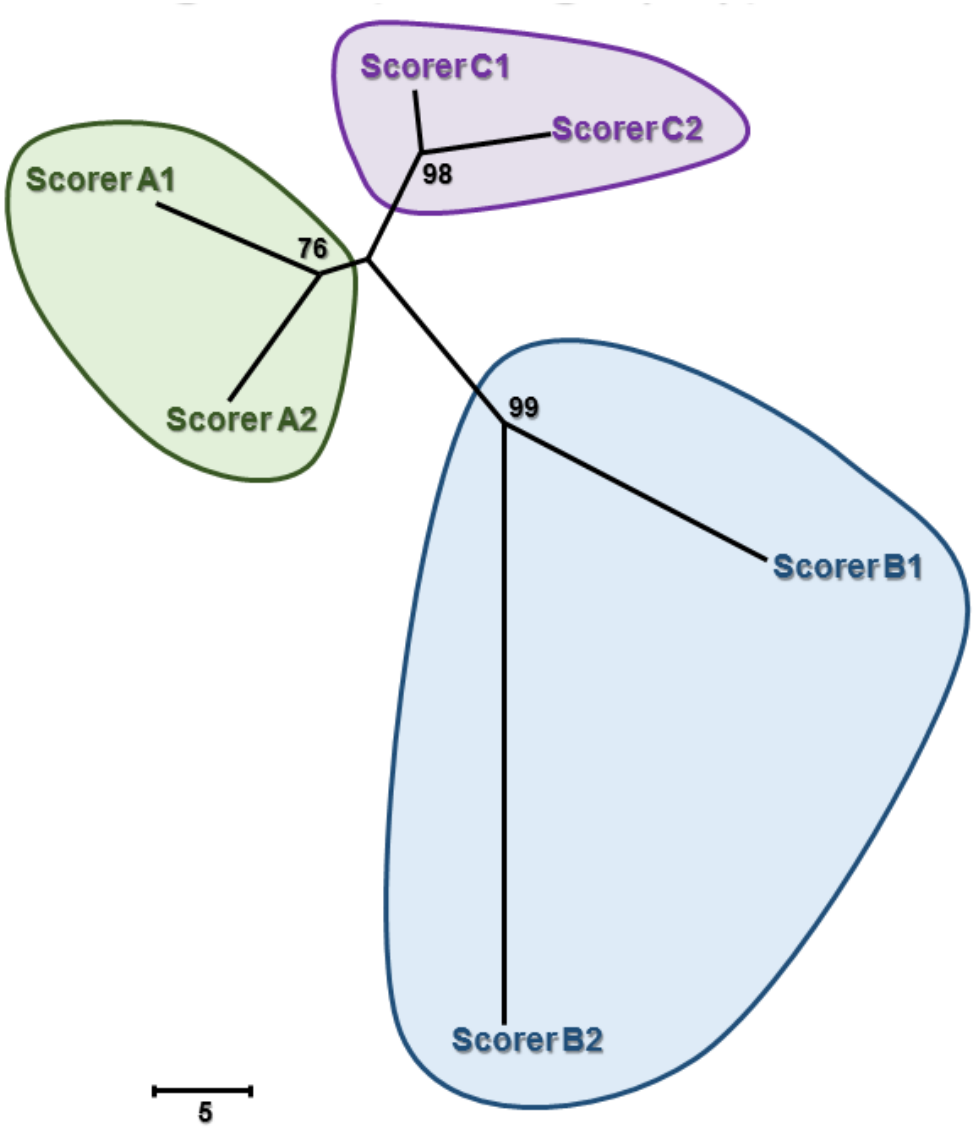

Figure 3. Phylogram demonstrating the agreement within and between scorers for radiographic scoring of IVD calcification. The length of the branches between different scorers (A, B, C) represent the disagreement between scorers. The length of the branches between two scorer iterations (1, 2) represents the within-scorer disagreement. The scale is based on the number of differing scores out of the 544 IVDs assessed by an individual scorer. Numerical bootstrap values indicate strength. Scale bar = 5 IVD scoring differences.

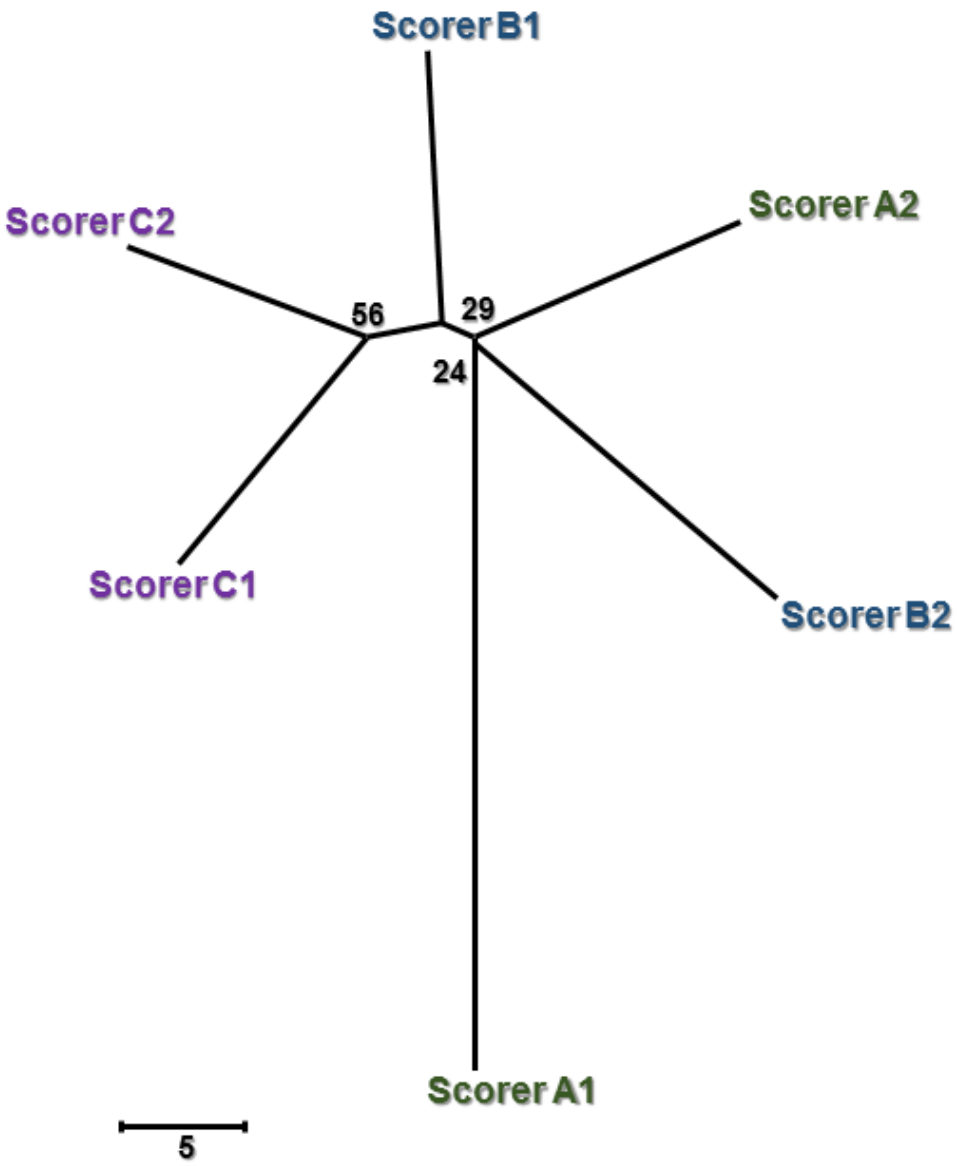

Figure 4. Phylogram demonstrating the agreement within and between scorers for computed tomographic (CT) scoring of IVD calcification. The length of the branches between two scorer iterations (1, 2), and between each of the three scorers (A, B, C), represents the within-scorer disagreement and between-scorer disagreement, respectively. The scale is based on the number of differing scores out of the 314 IVDs assessed by an individual scorer. Numerical bootstrap values indicate strength. Scale bar = 5 IVD scoring differences.

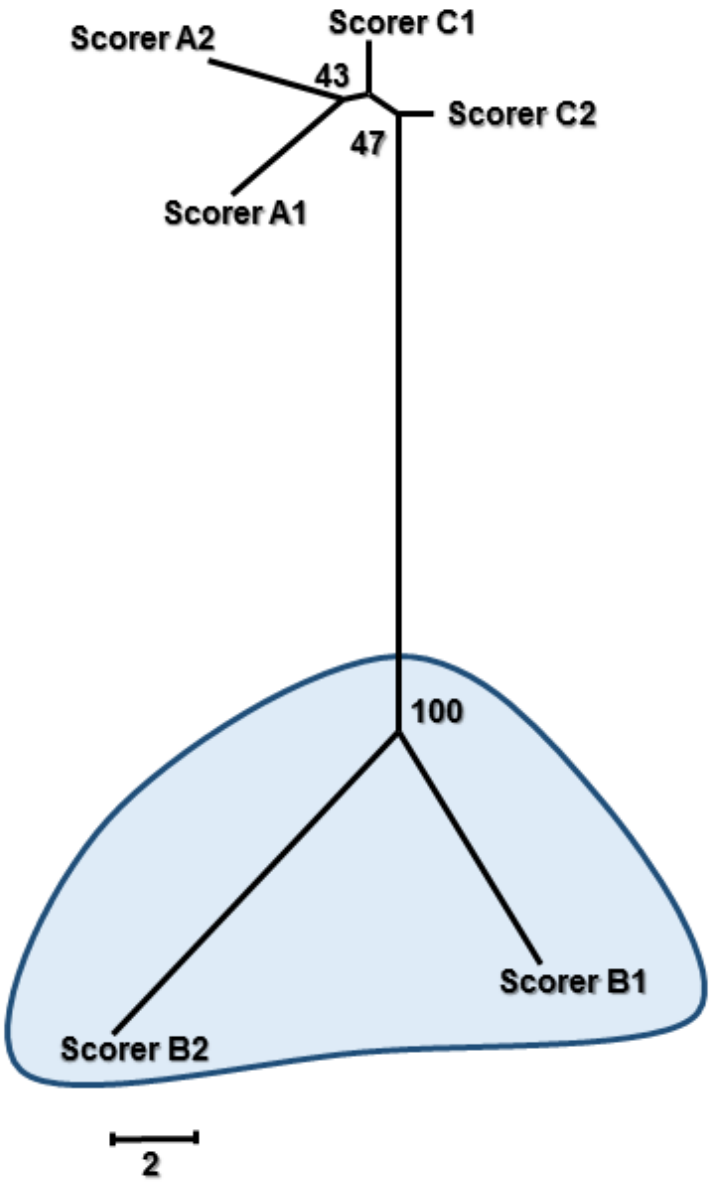

Figure 5. Phylogram demonstrating the agreement within and between scorers for magnetic resonance imaging (MRI) scoring of IVD calcification. The length of the branches between two scorer iterations (1, 2), and between each of the three scorers (A, B, C), represents the within-scorer disagreement and between-scorer disagreement, respectively. The scale is based on the number of differing scores out of the 142 IVDs assessed by an individual scorer. Numerical bootstrap values indicate strength. Scale bar = 2 IVD scoring differences.

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Conclusion

Intervertebral disc disease (IVDD) is widely accepted as a major problem in the Dachshund breed, imposing high levels of morbidity and mortality on affected dogs and challenging, often financially constrained decisions around treatment options on pet owners. Therefore, continued research into this disease and possible methods of reducing its occurrence is important.

Supporting historical data, a recent investigation again identified a high prevalence of IVDD in a primarily UK-based Dachshund population, reporting a prevalence of 15.7% overall but a range of 7.1% to 24.4% depending on the breed variant[1]. A mixture of genetic, physical and lifestyle-related factors likely contributes to this high prevalence figure; for example, lifestyle factors associated with a higher risk of IVDD in that study included reduced daily exercise and activity levels, and not being allowed to jump on/off furniture[1]. Because Dachshunds have chondrodystrophy, a disorder of cartilage formation, their intervertebral discs undergo accelerated degeneration which can include calcification in advanced stages. The number of calcified intervertebral discs present in a Dachshund spine at a young adult age is both highly heritable and a good predictor of the future likelihood of experiencing clinical IVDD, and can be detected radiographically. These factors can be used advantageously in selective breeding programs. As such, a radiographic spinal screening program was developed and implemented, initially in several Scandinavian countries. The total number of radiographically detectable intervertebral disc calcifications (RDIDC) within an entire dog spine is measured and recorded as a total discrete numerical score between 0 and 26. The screening tool is now also used sporadically around the world, including in Australia. However, at the commencement of this project, widespread global knowledge of the scheme was lacking, and there was not a single report summarising the scheme

and its underlying scientific basis within the literature. Scorer variability (i.e. precision) in RDIDC scoring had not been evaluated, and further, alternate imaging modalities had not been assessed for their potential utility in scoring Dachshunds for intervertebral disc calcification.

Through this project, we aimed to address some of the identified deficiencies in the literature and progress the already established screening program forward. The results of this body of work have contributed to the growing knowledge around canine IVDD and RDIDC scoring in Dachshunds. A comprehensive review of radiographic scoring for intervertebral disc calcification in the Dachshund is now available in the literature. This review appraised and presented a summary of the available literature on the topic, confirming the underlying basis for the development of the radiographic screening scheme. Moreover, the review identified areas warranting further investigation.

As alluded to in the review, the effectiveness of the radiographic screening program to reduce the incidence of IVDD in Dachshunds had not yet been thoroughly analysed. However in recent years, a few studies have begun to explore the relationship between clinical IVDD and morphological intervertebral disc changes as identified with diagnostic imaging[2-4]. One paper confirmed that RDIDC (especially scores ≥ 5) and the development of clinical IVDD were strongly associated with one another, and that spinal radiography is an appropriate tool for screening breeding dogs to select against RDIDC and IVDD[2]. These authors found high heritability estimates for the number of calcified IVDs in Finnish Dachshunds, and identified small genetic improvements that had been made over the 15-year period during which the RDIDC scoring scheme has been in use, thus recommending systematic radiographic screening for IVD calcification in breeding Dachshunds[4]. Meanwhile, Kranenberg et al. (2013) evaluated the relationship between intervertebral

disc degeneration as graded on MRI using the Pfirrmann grading system and the clinical severity of neurologic signs associated with IVD herniation[3]. They found that there was a significant correlation between MRI and histological grading of intervertebral disc degeneration, supporting prior results[5]. However, neither MRI or histological grading were significantly correlated with the severity of clinical neurological signs, leading the authors to suggest that neural compression, in addition to degeneration grading, should form part of an MRI scoring system for clinical cases of IVDD[3].

Identified knowledge gaps from the literature review guided our experiments. The results of our first experiment showed that using spinal radiographs as a screening tool for intervertebral disc calcification in Dachshunds has an overall high level of precision (within- and between-scorer agreement) regardless of scorer experience, making it a reliable method. However, some degree of scorer subjectivity exists, and improved precision is achieved by veterinarians with more experience interpreting spine radiographs. It was therefore recommended that scoring be performed by such experts, with training and/or experience as prerequisites to becoming an RDIDC scorer. Further, due to the inherent limitations of radiographs resulting from anatomic superimpositions, the investigation of an alternative screening test utilising a cross-sectional imaging modality (such as CT) was recommended.

Through collaboration with researchers at the University of Helsinki in Finland, a country in which RDIDC screening has been in effect for many years, we collected data that allowed us to begin analysing CT and MRI for their potential use in IVD calcification screening. The results of this second experiment reinforced those from the first, identifying radiography as a highly precise test for IVD calcification scoring, with some degree of scorer subjectivity present. Precision levels were even higher than in the first experiment, attributable to the greater radiographic interpretation

experience of the scorers in this study. At face value, there was no evidence to suggest that CT or MRI should replace radiography as a screening test because both modalities showed lower levels of scorer agreement, with the lowest estimates seen for CT. However, the anecdotal clinical suspicion that a greater number of calcified intervertebral discs would be detected using CT compared to radiography and MRI was supported by our experimental results. This is likely because of increased sensitivity of the modality due to its improved contrast resolution and cross-sectional nature, which reduces issues of superimposition and allows for multiplanar reconstructions. Also, individual scorer variability (i.e. subjectivity) was relatively low for CT, with the repeatability approximating the reproducibility. The lower scorer agreement for scoring IVD calcification using CT may have, in part, been influenced by the higher overall number of detected IVD calcifications with potentially lower scorer confidence when scoring small / questionable calcifications, resulting in greater variability between scoring iterations.

Based on the findings reported herein, use of the established radiographic screening program for intervertebral disc calcification in Dachshunds is supported and recommended. Greater education about the scheme is needed to facilitate wider adoption of the tool, both within the veterinary profession and the Dachshund breeder community. This is particularly relevant in countries where the program is not well known or readily used. The relatively small number of RDIDC scorers worldwide also potentially limits the scheme, and the development of a training tutorial/certification process for interested veterinarians could be considered. As with any screening tool, if it is not widely used to inform breeding decisions and impact the gene pool, substantial progress towards disease reduction will not be made[4].

Many of the initial aims generated at the outset of this project have been achieved. Despite the work done, further investigation is warranted. Outstanding is a more in-depth review of the data obtained from our second experiment. Expanding on the analyses performed in Chapter 3, we will evaluate the performance of the radiographic, CT and MRI tests in terms of accuracy (i.e. specificity and sensitivity). Moreover, we plan to perform additional analyses of precision and accuracy for specific regions of the spine that are particularly challenging to score radiographically due to superimposition of anatomy (e.g. cervicothoracic junction). Furthermore, we would like to review how RDIDC scoring performs based on the size of intervertebral disc calcifications (big vs. small), by different spine regions (e.g. cervical vs. thoracic vs. lumbar) and by individual intervertebral discs (e.g. does scoring individual discs independent of the whole spine alter the results?). The results gained will inform and optimize breeding recommendations.

As the radiographic tool will likely prevail as the widely-used option for intervertebral disc calcification screening in veterinary practice due to its superior availability and cost effectiveness compared to CT and MRI, the potential effect of instruction and training on individual scorer subjectivity is of interest. We propose an additional experiment using novice veterinary students who do not have prior experience scoring RDIDC or any other pre-existing biases. The students would be divided into two groups, with group 1 students being asked to score Dachshund spine radiographs for RDIDC without any instruction on how to do so, and group 2 students asked to score the same radiographic studies after receiving a tutorial on how to perform the scoring. The aim would be to assess whether structured training impacts scoring outcomes, and could alter the recommendation that only expert veterinarians perform the scoring. Aspects of self-learning could also be evaluated if the students were asked to (blindly) score duplicate or triplicate studies over various time intervals.

Similar to the study with the Finnish Dachshund population[4], a final aspect of this research would be to perform an epidemiological study of breeding Australian Dachshunds to establish baseline population statistics such as prevalence of intervertebral disc calcification, median RDIDC score, and potential correlations with coat or size variants. This database, in conjunction with information about breeding lines and related individuals, could allow monitoring of improvement in RDIDC scores and clinical IVDD in the breeding dog population following use of the screening program. To date, more than 70 Dachshunds with RDIDC scores are in the database.

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