CANINE HIP AND ELBOW DYSPLASIA IMPROVEMENT PROGRAMS AROUND THE WORLD: SUCCESS OR FAILURE?

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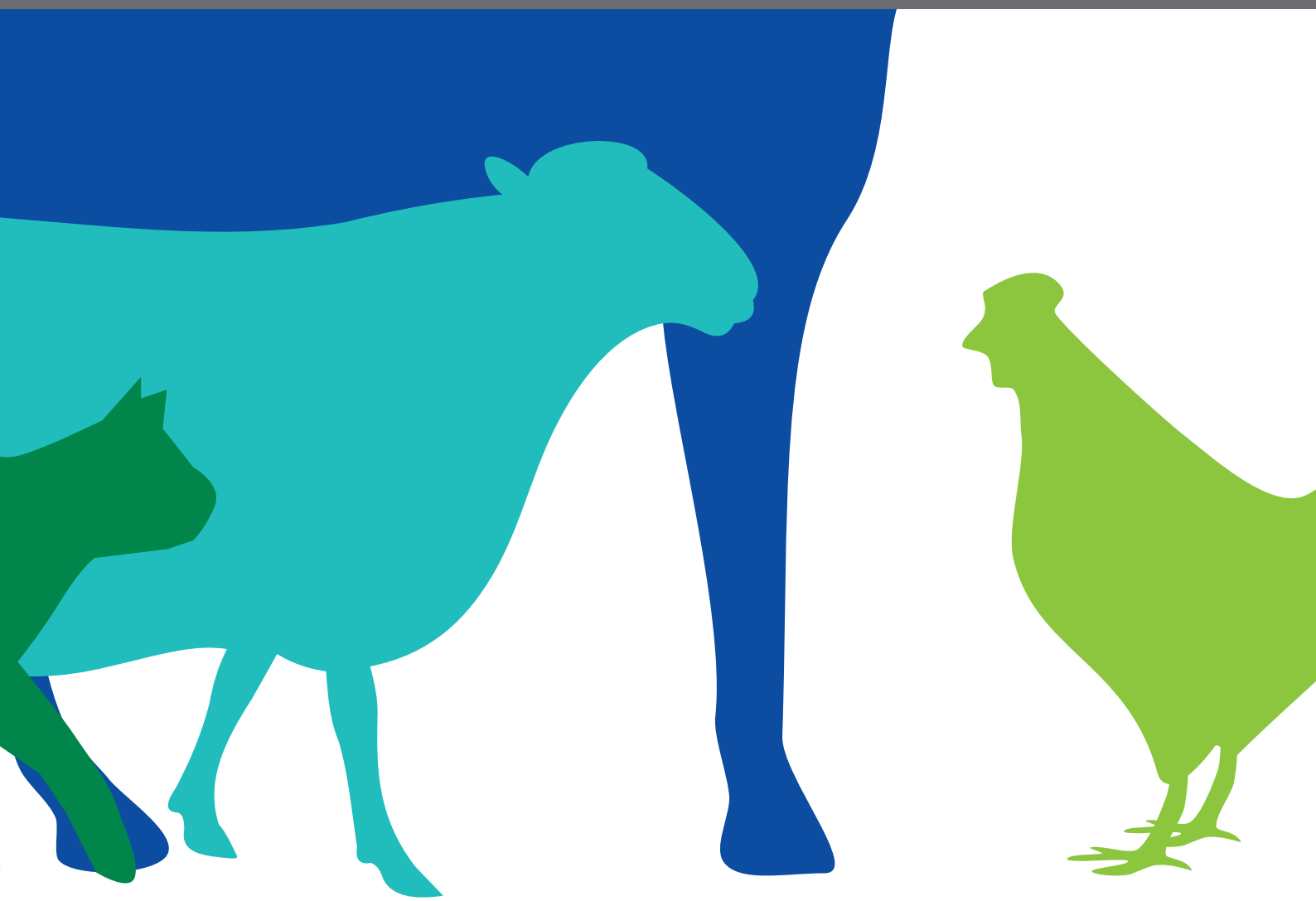

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CANINE HIP AND ELBOW DYSPLASIA IMPROVEMENT PROGRAMS AROUND THE WORLD: SUCCESS OR FAILURE?

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EDITORIAL
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Editorial: Canine Hip and Elbow Dysplasia Improvement Programs Around the World: Success or Failure?

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Keywords: hip dysplasia, elbow dysplasia, breeding value, genomic selection, hip laxity

Editorial on the Research Topic

Canine Hip and Elbow Dysplasia Improvement Programs Around the World: Success or Failure?

This compilation of 13 papers under the Research Topic "Canine Hip and Elbow Dysplasia Improvement Programs Around the World: Success or Failure?" addresses different aspects of the research that is currently being done about canine hip dysplasia (HD) and elbow dysplasia (ED), giving a special emphasis to the analysis of HD and ED in some canine populations over time. We can say that hip HD first, and then ED have been responsible for a real headache for veterinarians and dog owners for the past 50 years and an important limitation on the welfare of affected dogs. Both diseases continue to have a high prevalence, especially in some large and giant canine breeds.

Recognition of hereditary HD etiology coincides with the first descriptions of the disease. However, knowledge in the form of inheritance has evolved from a simple Mendelian inheritance to a polygenic and multifactorial nature.

The strategy to deal with canine HD and ED was based on two essential pillars, with the aim, on the one hand, of selecting animals without the disease for breeding to reduce the frequency of bad genes in the population, and, on the other, of treating the individual affected animal to improve their well-being. In this aspect, radiographic examination has been used since the 1960's for HD diagnosis and for selection of animals for breeding with the aim of eradicating the disease (1). The diagnosis of HD on radiographs has been grounded in the detection of signs of degenerative joint disease. These aspects were well-highlighted in the reviews by Hedhammar and Anderson et al. presented in this collection of papers. In these early days, the selection of animals for breeding was based on the individual phenotype and the main aims were to reduce/eradicate HD prevalence. Many control programs fell rather short of what was expected, which led to some disbelief, mainly on the part of the breeders (2). Dogs with a normal phenotype may nevertheless be carriers of genes related to hip dysplasia. Since the 1980's, HD radiographic diagnostic methods based on passive hip laxity have also been developed. Hip laxity (HL) is considered the major HD risk factor. Hip laxity allows us to know the predisposition for HD earlier, at 4 months of age, the examination requires the use of a hip distractor. The use of HL is more popular in the United States, but recently two hip distractors have appeared in Europe, the Vezzoni and the DisUTAD. The latter is presented in this collection by Santana et al. (a), and it is currently being used in Portugal and Spain, and it is expected that it will become more widely used.

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Most HD and ED radiographs are obtained with the examiner's physical restraint of the dog on the X-ray table. However, precautions must always be taken to reduce the potential human exposure to the harmful effects of ionizing radiation. In this collection, Santana et al. (b) Present a paper in which HD radiographs were obtained using a hindlimb holder device, which allows HD images of high technical quality without any examiner help and without human exposure to ionizing radiation within the x-ray room.

A recent reality in terms of radiographic images was the introduction of the digital image. On the one hand, it can be an asset in the correction and optimization of some lower-quality images resulting from inappropriate x-ray beam exposure factors. But on the other hand, they are also subject to specific quality aspects that, if not properly understood, may result in inadequate radiographic diagnoses. Some of these aspects are well-illustrated in relation to HD and ED in this collection in papers by Moorman et al. and Válega et al. respectively.

Elbow dysplasia is especially important in the dog, while HD is also clinically very important in humans. The strategy with respect to human HD has always been focused on diagnosis and treatment, and this has been a great success, both in respect to disease prevention and to treatment. In veterinary medicine, the success is also very satisfactory in terms of therapeutic options for advanced disease with joint destruction, where we highlight the success of treatments with complete total hip replacement (3). For the elbow joint, the total replacement option is not so common. Some basic research in this area is still needed. Aiyan et al. present a paper to study the proximal femoral morphology. For severe HD or ED cases, other therapeutic options, some surgical and others non-surgical, that are more conservative and less expensive remain available. In the papers by Silva Júnior et al. and Kriston-Pál et al., the administration of intra-articular substances is described. However, there is clearly a gap in veterinary medicine; it is the absence of a truly early diagnosis and of preventative complementary management methods that both reduce the severity of HD, and are easily accessible to all dogs, even mild forms of the disease. We believe that mild forms of disease, even if dogs do not show evident clinical signs, may be associated with discomfort and reduced animal welfare. In this regard, the approach to HD management in terms of human medicine is clearly more developed. In humans, the diagnosis of developmental dysplasia is confirmed at birth, since in this case HD has the peculiarity of being congenital, and preventive management is recommended for all situations. As for the early diagnosis of HD in dogs, it usually takes place at about 4 months of age and preventive treatment is usually reserved for animals with clinical signs of the disease, we think that there are great possibilities for an optimization approach here. Furthermore, current surgical treatments in canine HD involve altering the normal pelvic bone structure of the animal, and the response to the most severe forms of the disease is not always the best.

Based on the evolution of knowledge on the control of other inherited polygenic and environmental characteristics, such as the production of milk and meat in farm animals, the control of HD and ED in dogs was redirected. The reference ceased to be the individual phenotype and became the breeding value. The selection of dogs by HD breeding values requires HD databases with the largest number of animals possible. Breeding values are a more reliable reference of the genetic component of the animals for the disease, and the current results are considered very promising; these aspects are well-documented in this collection in the papers of Wang et al., and James et al. Therefore, the current recommendation for the control of hip and elbow dysplasia is to use breeding values as the main selection criteria. Some studies to better understand the form of HD and ED heritability continue to be carried out; Baers et al. presents a paper on this subject. However, the use of individual phenotypes for animal selection in control programs continues to be used in some countries with success in reducing prevalence and severity in canine populations (Ohlerth et al.). We think that in this area, there is now sufficient knowledge and means to deal with HD and ED diseases effectively.

However, currently the goal is the evolution toward genomic selection, which is already a present reality in farm animal milk and meat production and allows animal selection without the need for phenotype population databases. Genomic selection has been based on the detection of single nucleotide polymorphisms associated with genetic characteristics. Hip dysplasia is also taking the first steps in this direction. A molecular test has already been made available, the Dysgen, although it has not achieved the desired success (4). An obvious advantage of this approach in the future is that there is no need for a radiographic examination and all associated medical procedures.

In response to the question of the topic, we believe that over these 50 years the successes and advances made in the knowledge of HD and ED have been very significant and have undoubtedly eventually overcome failures that may have existed. It is evident that there has been an evolution in the strategies that have been used over time to deal with HD and ED, both in terms of treatment and in the selection of animals for breeding. But it is also notable that in both aspects related to these health areas, additional improvements are possible based on more targeted research. We hope that this collection of research articles will serve to increase awareness of current activity in this area and point to possible potential improvements and developments in the fields of hip and elbow joint dysplasia.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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ORIGINAL RESEARCH
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Prevalence of Canine Hip Dysplasia in Switzerland Between 1995 and 2016—A Retrospective Study in 5 Common Large Breeds

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Canine hip dysplasia (CHD) is a genetic disease, modulated by epigenetic and environmental factors. To decrease the prevalence of CHD, the hip joints of many pure breed dogs are radiographed to identify dysplastic dogs not qualified for breeding. It has been shown that both, prevalence and severity of CHD may be reduced on the basis of phenotypic i.e., radiographic selection of breeding animals. The method has been used in many countries for over 50 years. In the present study, severity and prevalence of CHD in five common large breeds in Switzerland were evaluated since 1995. Both, prevalence and severity of CHD dropped in each breed between the periods 1995–1999 and 2010–2016. The prevalence decreased in Golden Retrievers from 25 to 9% and in Labrador Retrievers from 16 to 3%, respectively. In the Flat-Coated Retriever, prevalence in general was low, decreasing from 6 to 3%. In the Bernese mountain dog and the German shepherd dog, a decrease from 21 to 12% and from 46 to 18%, respectively, was observed. However, the rather low overall rate of radiographed dogs (Retrievers: 11 to 18%, Bernese Mountain dogs: 23% and German Shepherd dogs: 31%) does not allow to draw reliable conclusions regarding the true prevalence of CHD for the entire population of these breeds in Switzerland.

Keywords: Bernese mountain dog, dog, canine hip dysplasia, German shepherd dog, prevalence, retriever

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INTRODUCTION

Canine hip dysplasia (CHD) is defined as a developmental disease of the coxofemoral joint (1) and occurs in most canine breeds. The joint appears normal and congruent at birth but develops abnormal during growth. Excessive laxity is assumed to be the key factor leading to subluxation of the femoral head, incongruity of the joint and subsequent flattening of the acetabulum (2). As a result, the load on the cartilage is unevenly distributed resulting in unequal cartilage abrasion, followed by an inflammatory response and finally secondary degenerative joint disease, causing pain and lameness (1, 3). Presence and degree of CHD may be assessed on the basis of radiographic changes, i.e., subluxation, joint deformation, and osteoarthritis (4).

Canine hip dysplasia is a multifactorial disease triggered by genetic, environmental and probably epigenetic factors (5, 6). The genetic basis of CHD is not fully understood; however, it is assumed to be a complex genetic trait with a polygenic inheritance pattern. Both, dominant and recessive modes of inheritance have been discussed (7, 8). Due to its genetic background, excluding affected

dogs from breeding may reduce the prevalence of CHD. The heritability of CHD and therefore the response to selection is breed dependent. The higher the heritability of a trait, the greater is the expected genetic improvement over time when selective breeding is practiced (9, 10). Breeding stock is usually selected based on the radiographic phenotype.

The radiographic projection in dorsal recumbency with extended hip joints is used for assessment of CHD in most countries (11). In Switzerland, a second view with flexed and abducted stifles is mandatory for official scoring to improve scoring quality (12). Minimum age of the dogs for official scoring is between 12 and 24 months, depending on the country and the scoring method used.

Prevalence of CHD has been reported to vary significantly between breeds and countries. In France, the prevalence varied between 3.9% (Siberian Husky) and 59.7% (Cane Corso) over the period 1993–2006 (13). In the United States, values of 1.5% in the Miniature Schnauzer and 35.4% in Rottweilers were reported between 1991 and 1995 (14). In Switzerland between 1991 and 1994, the CHD-prevalence ranged from 7% in Siberian Huskies to 69% in Gordon Setters. For popular breeds such as the Retrievers, the Bernese mountain dog and the German shepherd dog, prevalence of CHD was in the range of 31–53% (15). During the last decades many selective breeding programs based on the radiographic scoring have been implemented for different breeds with the aim to reduce the prevalence and severity of CHD, and consequently, to improve animal's welfare. A decrease of the CHD-prevalence was noted in some reports (13, 16, 17); however, progress was slow or inexistent in others (18, 19).

Reports of the recent prevalence of CHD in Switzerland are lacking in the peer-reviewed literature. The aim of the present study was therefore to assess the overall prevalence of CHD and its change since 1995 in five common large dog breeds in Switzerland.

MATERIALS AND METHODS

Animals

The largest purebred dog populations in Switzerland i.e., Golden Retrievers, Labrador Retrievers, Flat Coated Retrievers, Bernese Mountain dogs and German Shepherd dogs were included in the study. The Swiss kennel clubs provided the official CHD score and the date of birth for each dog. The vast majority of the dogs were examined in their second year of life.

TABLE 1 | Comparison of scoring protocols for canine hip dysplasia.

Switzerland (point score per joint)	FCI	BVA/KC (point score per joint)	OFA
0–2	A: normal	0–3	Excellent
3–6	B: near normal	4–8	Good
7–12	C: mild CHD	9–18	Fair, borderline, mild
13–18	D: moderate CHD	>18	Moderate
>18	E: severe CHD		Severe

FCI, Fédération Cynologique Internationale, BVA/KC, British Veterinary Association/The Kennel Club, OFA, Orthopedic Foundation for Animals.

Scoring Protocols

Most dogs were scored according to the Swiss scoring system (4). Ninety-six imported dogs had been scored abroad; for the present study their scores were transferred to the Swiss system as shown in **Table 1**.

The Orthopedic Foundation for Animals (OFA) system is used in the USA and Canada. Minimum age for scoring is 2 years. Seven grades are defined: excellent, good, fair, borderline, mild, moderate, or severe. The borderline grade is assigned to incongruent joints of undetermined quality but without degenerative changes. The British Veterinary Association/The Kennel Club (BVA/KC) system is used in Britain, Ireland, Australia and New Zealand. Dogs older than 12 months are evaluated. Nine radiographic criteria are evaluated; each rated with 0–5, or 0–6 points, respectively (20, 21). A total between 0 and 52 points is allotted to each joint. In Britain, the points per joint are added up representing the final score whereas in Australia only the total points of the worse hip joint is used for the final score (22). The Fédération Cynologique Internationale

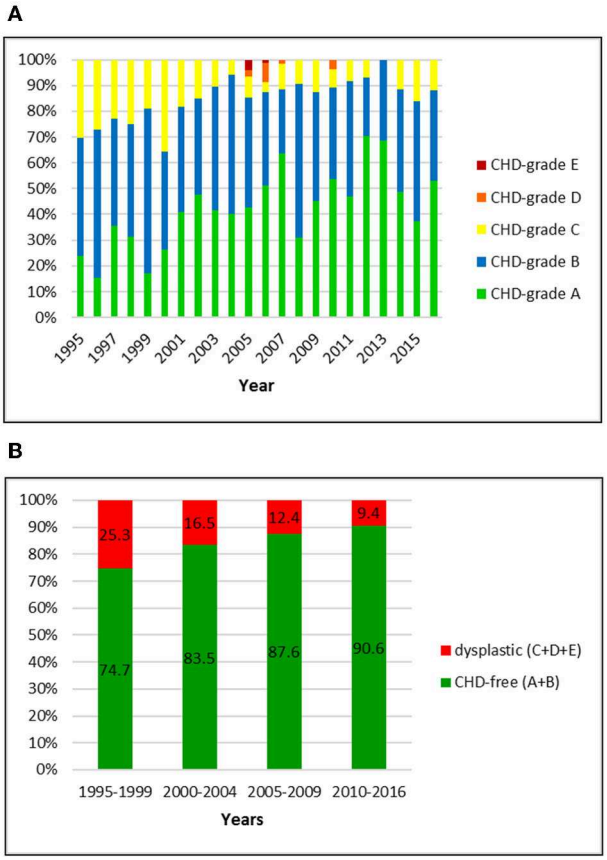

FIGURE 1 | (A) Distribution of CHD-grades in the Swiss Golden Retriever population from 1995 to 2016. **(B)** Proportion of dysplastic and CHD-free Golden Retrievers in Switzerland from 1995 to 2016: the prevalence of CHD dropped markedly over 22 years.

(FCI) system is used in most European countries, Russia, South America, and Asia (23). Minimum age for official scoring is 12 months, in giant breeds 18 months (24). Each joint is allotted to one of five grades (A–E) that are defined descriptively; the final grade refers to the worse joint. In Switzerland a system is used linking the British system with the FCI grading system allowing a more systematic and objective scoring. The same six radiographic criteria as in the FCI system are evaluated; each criterion is allotted 0–5 points leading to a sum of 0–30 points per joint. Criteria 1 and 2 quantify the degree of laxity. Criteria 3 and 4 determine modeling of the acetabulum and criteria 5 and 6 include arthritic changes of the femoral head and neck (4). The numeric score is then translated into the FCI-grades A–E (23). For the Bernese mountain dog the minimal age for official scoring in Switzerland is 14 months, whereas it is 12 months for the Retriever breeds and the German shepherd dog.

Statistical Analysis

The kennel clubs provided data on birth rate and official CHD score. Descriptive statistics was performed using the SPSS statistics program (Version 19, IBM Corporation, Armonk, New York). Hip dysplasia grades A and B were considered normal joints (CHD-free) whereas the grades C, D, and E were considered dysplastic.

RESULTS

Prevalence of the five CHD-grades and prevalence of dysplastic dogs are shown for each breed from 1995–2016 in **Figures 1–5**.

Golden Retriever

The Swiss retriever club registered an official CHD-score for 1047 Golden Retrievers between 1995 and 2016. Of these dogs, 27 (2.6%) were scored abroad (BVA/KC system). The overall prevalence of CHD was 15.8% including 14.4, 1.0, and 0.4% dogs with grade C, D, and E, respectively. Of the 84.2% CHD-free dogs, 41.6% were scored grade A and 42.6% grade B, respectively. In the first period (1995–1999), 25.3% of the Golden Retrievers were dysplastic whereas in the final period (2010–2016) the prevalence of CHD had dropped to 9.4%. Most remarkable were the decrease of dogs with grade C (from 25.3 to 9.0%) and the increase of A-scored dogs (from 24.1 to 53.5%). The fraction of A and B joints reached ∼90% in 2004 and remained unchanged ever since.

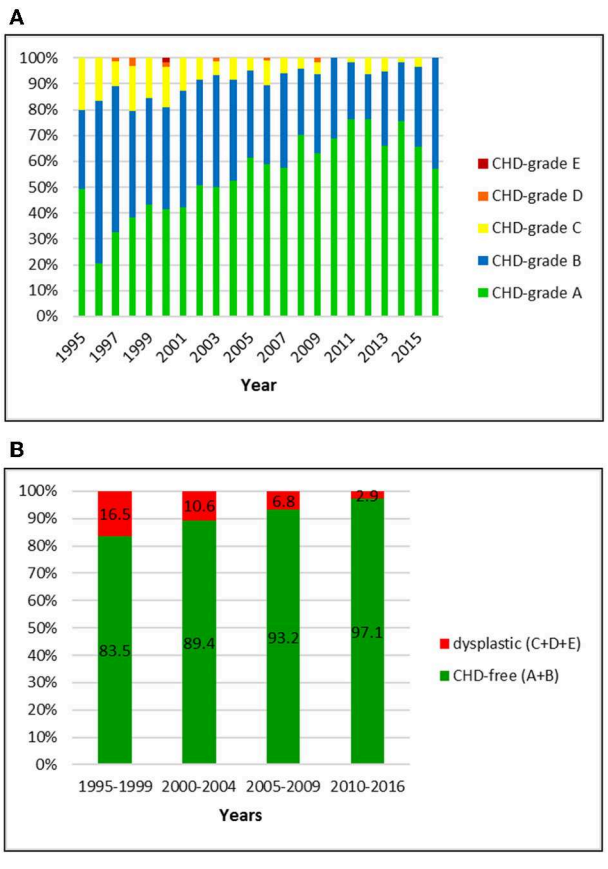

FIGURE 2 | (A) Distribution of CHD-grades in the Swiss Labrador Retriever population from 1995 to 2016. **(B)** Proportion of dysplastic and CHD-free Labrador Retrievers in Switzerland from 1995 to 2016: the prevalence of CHD decreased over 22 years.

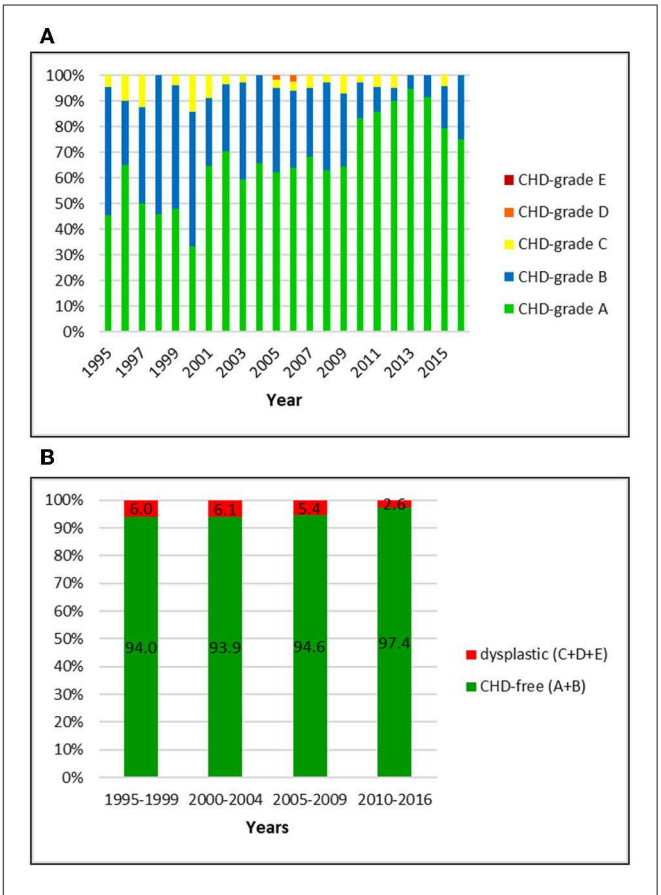

FIGURE 3 | (A) Distribution of CHD-grades in the Swiss Flat-Coated Retriever population from 1995 to 2016. **(B)** Proportion of dysplastic and CHD-free Flat-Coated Retrievers in Switzerland from 1995 to 2016: in comparison to the other breeds, the prevalence of CHD has been markedly lower and values stayed constant during the study period.

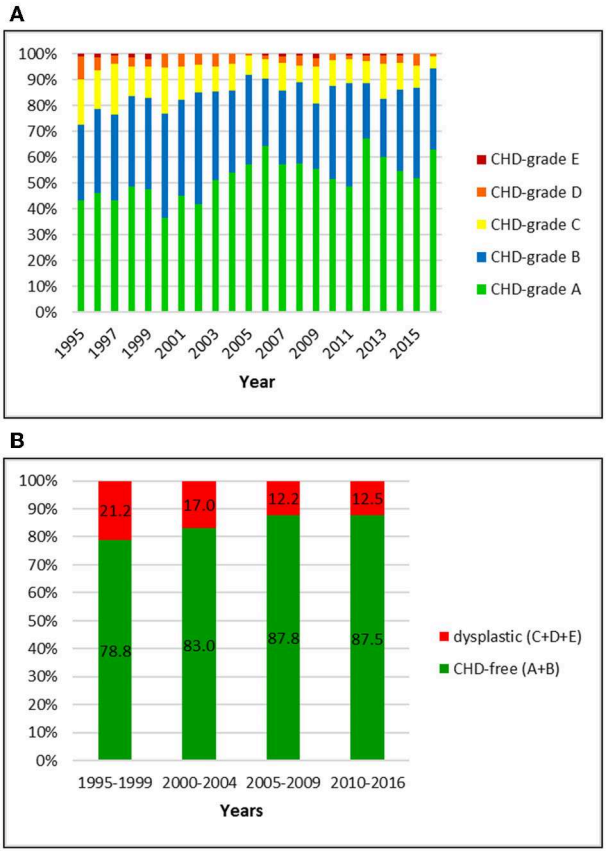

FIGURE 4 | (A) Distribution of CHD-grades in the Bernese mountain dog population in Switzerland from 1995 to 2016. **(B)** Proportion of dysplastic and CHD-free Bernese mountain dogs in Switzerland from 1995 to 2016: the prevalence of CHD mildly decreased over 22 years.

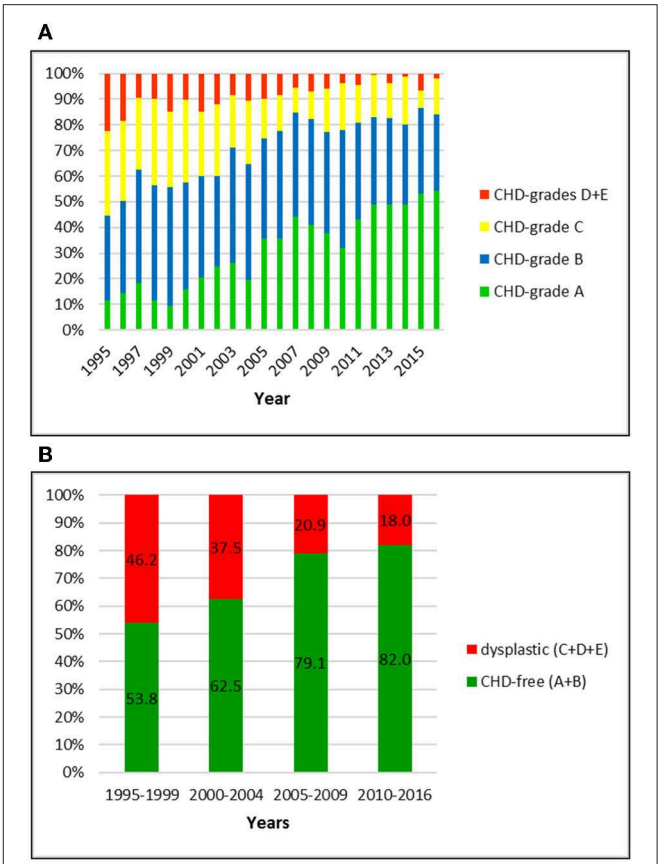

FIGURE 5 | (A) Distribution of CHD-grades in the German shepherd dog population in Switzerland from 1995 to 2016. **(B)** Proportion of dysplastic and CHD-free German shepherd dogs in Switzerland from 1995 to 2016: the prevalence of CHD was initially the highest and showed the largest decline over 22 years compared to the other investigated breeds.

Labrador Retriever

Official data of 1512 Labrador Retrievers were available for the period from 1995 to 2016. Of these, 51 (3.4%) had a BVA/ KC-score and six (0.4%) dogs an OFA-score that was translated into the Swiss system. The overall prevalence of CHD was 9.1%, including 8.5, 0.5, and 0.1% of dogs with grade C, D, and E, respectively. An A-score was allotted to 54.6% and grade B to 36.3%. In the initial period, 16.5% were scored dysplastic. The number dropped to 2.9% in the latest period 2010–2016. There was a remarkable decrease of C-rated dogs (15.7–2.9%) and an increase of A-rated dogs (36.8–70.6%). More than 90% of the dogs were CHD-free since 2006.

Flat-Coated Retriever

Overall, 768 Flat-Coated Retriever with an official CHD-score were registered by the Swiss Retriever Club between 1995 and 2016, of which three (0.4%) were BVA-rated and then translated into the Swiss system. The overall CHD-prevalence of 5.0% included 4.6% with grades C, 0.4% with grade D and 0% with grade E, respectively. The proportion of CHD-free dogs was 95.0%; of these, 65.7% were rated grade A and 29.3% grade B, respectively. The proportion of dysplastic dogs was initially 6.0% and dropped to 2.6% in the period 2010–2016. Simultaneously, the proportion of C-rated dogs dropped from 6.0 to 2.6% and the percentage of A-rated dogs increased from 50.0 to 86.6% in these years. Responsible for this noticeable increase of A-rated dogs was not only a reduction of C-dogs but also a decrease of B-rated dogs: from 44.0% in 1995–1999 to 10.8% in the final period.

Bernese Mountain Dog

The Swiss Club for Bernese Mountain Dogs registered 3,381 dogs with an official CHD-score between 1995 and 2016. Nine (0.3%) dogs were BVA/KC-rated.

Dysplastic hip joints were noted in 15.7% of the dogs, including 11.6, 3.5, and 0.6% of dogs with grade C, D, and E, respectively. Of the CHD-free dogs (84.3%), 51.6% were rated as CHD-A and 32.7% as CHD-B. In the initial period (1995–1999) the CHD-prevalence was 21.2%. It dropped to 12.5% in 2010–2016. While C-rated hip joints decreased from 15.2 to 9.5%, A-rated dogs increased from 45.8 to 56.7% in these years.

German Shepherd Dog

Data of 5326 German shepherd dogs was available for the study period. All dogs were scored by the Swiss system. The overall

prevalence of CHD was 32.4%; of these, 22.5% were scored grade C and 9.9% grade D or E, respectively. Grade D and E were summarized in the database of the breeding club and therefore cannot be shown separately. The proportion of CHD-free dogs was 67.6%, including 27.9 and 39.7% of dogs with grade A and B, respectively. When compared to the other breeds, German shepherd dogs showed the highest prevalence of CHD initially followed by the steepest decline over 22 years. While 46.2% of them were dysplastic between 1995 and 1999, the number dropped to 18.0% in the period 2010–2016. In particular, the number of A-rated dogs increased from 13.0 to 46.5%, and the number of C-rated dogs decreased from 31.1 to 14.8%.

Scoring Rate

The rate of radiographed dogs was calculated based on the number of puppies born per year. Offspring statistics of the examined breeds were not available for the entire study period. In the Golden Retriever, 7,947 puppies were registered between 1997 and 2015. Of these 881 dogs were screened for CHD, representing a scoring rate of 11.1%. In the Labrador retriever, litter information was available for the period between 2003 and 2016. Of 6,155 dogs born, 866 (14.1%) were officially scored for CHD. The scoring rate for the 3819 Flat-Coated Retrievers registered between 1998 and 2015 was 17.3% (659 dogs). In the Bernese mountain dog, 12,565 puppies were born between 1997 and 2015 of which 2,863 (22.8%) underwent official CHD screening. The highest scoring rate was noted in the German shepherd dogs: 13,998 dogs were born between 1997 and 2015 and 4,327 dogs were officially scored, equalling a scoring rate of 30.9%. Scoring rate per year is shown for each breed in **Figure 6**.

DISCUSSION

Control of CHD in Switzerland started around 1965. Obviously dysplastic dogs i.e., score D or E have been banned from breeding in Switzerland since more than 50 years. Nevertheless, the CHD-rate remained very high for a long time reaching levels above 50% in some breeds. Between 1991 and 1994, the CHD-prevalence was

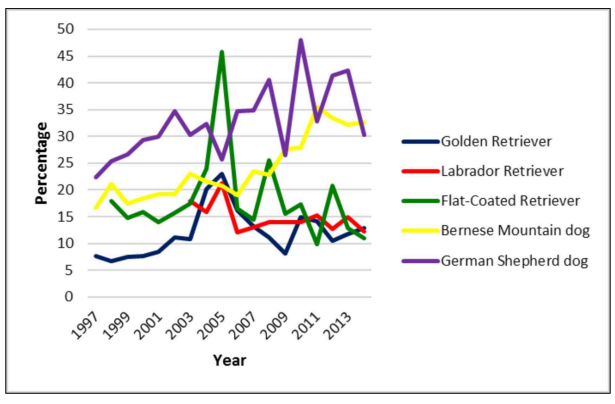

FIGURE 6 | Scoring rate in five common large breeds in Switzerland from 1995 to 2016. It was lowest in the Golden Retriever and highest in the German shepherd dog. However, overall scoring rate was low.

51% in Golden Retrievers, 42% in Labrador Retrievers, 31% in Flat-Coated Retrievers, 46% in Bernese mountain dogs and 53% in German shepherd dogs, respectively (15). Since then, between 1995 and 2016, the CHD-prevalence has dropped considerably in all five breeds. In particular, there was a remarkable decrease of C-graded dogs and, inversely, an increase of A-graded dogs in all breeds. Whereas, grades D and E occurred mainly in the Bernese mountain dog and the German shepherd dog during the first decade of the investigated period of the present study, these scores were very rare in the Retriever breeds. During the second decade, it is interesting to note that in the German shepherd dog a continuous mild improvement of the hip joint quality was observed whereas the CHD-prevalence was already on a constant low level in the other investigated breeds. In other countries a similar but less pronounced decrease of CHD-prevalence has been reported. In France a significant decrease of CHD in six of 15 breeds was noted, e.g., the overall prevalence dropped from 27% in 1993–1999 to 19% in 2000–2006 in the Bernese mountain dog (13). In comparison, the overall CHD-prevalence in the Swiss population of Bernese mountain dogs dropped from 21% in 1995–1999 to 12.5% in 2010–2016. CHD-prevalence in Switzerland started on a lower level and the reduction was more pronounced, but observation time was also longer compared to the French study.

In the United States, a study between 1993 and 2003 showed only mild to no improvement. The rate of dysplastic Bernese mountain dogs dropped from about 16–12%, that of Labrador Retrievers from 12 to 9.5% and that of the Golden Retrievers from 18 to 15.5%, while the rate for German shepherd dogs oscillated between 11 and 19% with no clear improvement. Scoring rate was 5–7% for Retrievers and German shepherd dogs while it was roughly 24–34% for Bernese mountain dogs (25). In Finland no improvement was noted in Golden Retrievers, Labrador Retrievers and German shepherd dogs between 1983 and 1998 (19). In South African Labrador Retrievers only a minor improvement was seen between 2007 and 2015 (26). It was beyond the scope of the present study to investigate variables leading to a decrease in CHD-prevalence in the investigated breeds; however, several hypotheses may be discussed. Cross-national general factors may be responsible such as increasing awareness for inherited diseases by the kennel clubs, breeders and dog buyers on the one hand and improved training i.e., higher qualification of the scrutineers on the other hand. Vice versa, the lack of breeding restrictions associated with a lower scoring rate may be, in part, responsible for a lower progress in other countries when compared to the results of the present study. In Switzerland, for example, pairing C-graded dogs has been banned 10 years ago in German shepherd dogs, and in the Bernese mountain dog club, C-dogs may only be paired with A-dogs (https://www.retriever.ch/de/zucht/ankoerung; accessed July 21, 2019; https://www.bernersennenhund.ch/chronik; accessed July 21, 2019). Additionally, the Swiss registry may have helped to improve genetic hip quality because breeding dogs with better hips have been selected from the registered pool.

Furthermore, the scoring system in Switzerland is different from other countries. In all breeds, hip joint radiographs for official scoring are submitted to and evaluated by two

independent committees only. Additionally, the use of the point scoring system by Flückiger introduced in the early nineties (4) may have led to a more objective and stricter evaluation process. Differences in the prevalence and the course were also noted between breeds in the present study. Varying heritability, rate of imported breeding dogs with different genome, and overall breeding regimen e.g., selection for other genetic diseases may have played a role.

Currently a purely phenotypic selection mode against hip dysplasia is used in most breeding clubs worldwide. As long as no breeding restrictions are enforced it is very unlikely that the dysplasia rate of offspring can be lowered much further based on this modality alone. Further improvement may be expected by several approaches. The key methods are hip joint laxity measurement, calculation of estimated breeding values, rigid offspring control and genomic selection.

Hip joint laxity is considered the key factor for the development of CHD. The standard hip extended ventrodorsal projection masks hip joint laxity and the degree of laxity may be quantified by radiographic techniques such as the PennHIP method, the Fluckiger method or the dorsolateral subluxation test (27, 28, 28–30). Heritability estimates of the hip-extended score as well as hip joint laxity measurements have been shown to be high (31). However, according to a recent study, the Norberg angle was not an accurate predictor of canine hip conformation based on the distraction index and the dorsolateral subluxation score. Authors suggested that application of screening methods for CHD based on hip laxity (intermediate phenotype screening) would help to remove additional dysplastic dogs from the breeding pool (32).

However, the method is also confronted with multiple obstacles. PennHIP requires a special training and tool and submission of the radiographs to the company holding the copyright. Strict adherence to the proposed selection suggestions (27) would also result in gene loss as more than half of the tested dogs fail the test in some breeds. Breeders must control additional undesirable diseases as well such as elbow dysplasia, eye diseases, epilepsy, cancer, skin disorders and others. Breeders are also concerned about possible damage to the hip joints and therefore refuse implementation of a distraction technique. However, there is no data indicating that application of a distraction method affects the natural evolution of hip dysplasia and one method, the dorsolateral subluxation test (28), has been shown not to place additional stress on the canine hip above walking and running load.

Breed value estimation (BVE) has been proven to be highly successful in livestock such as cattle, swine, and poultry. Several studies estimated that the introduction of BVE in dogs imposes a more severe downward pressure on hip dysplasia prevalence than eye balling a pedigree (18, 20, 33–36). The estimated breeding value is calculated from the phenotype of an individual and its relatives and their pedigree relationship (21). Including information about the hip status of relatives has been proven to be an efficient selection mode in mass selection, as the mode of inheritance of CHD is still unclear and dogs with phenotypic normal hip joints may carry undesirable genes passing CHD to their offspring (21). The effect of BVE however is weak or even counterproductive when affected dogs are sorted out prior to official scoring, which is not uncommon practice in German shepherd dogs worldwide. To profit from BVE, a rigid unbiased offspring control should be installed. Breeding animals with poor quality offspring should be banned irrespective of their own hip status (37). Currently, offspring control rates are low, as radiography is quite expensive and deep sedation or general anesthesia is mandatory for the official radiological procedure. Owners are generally reluctant to have their dogs anesthetised as long as they are not intended for breeding and show no clinical signs.

Offspring control is an efficient way to reduce CHD. The Swiss School for Guide dogs for the Blind achieved impressive results after including offspring testing in their mass selection. The CHD-prevalence of their Labrador Retrievers dropped from 58% in the years 1972–1980 to 15% in the period 1991–1996 (38). The current dysplasia rate is <3%, and of these no D or E grades are noted (unpublished data, Dysplasia Committee Zurich 2016 and 2017). The school is scoring virtually 100% of the dogs and adheres to a strict selection scheme. Only dogs with CHD-grades A and B are used for breeding and information of the relatives are used when selecting potential breeding animals. Phenotypically normal sires are eliminated from the breeding stock if their offspring turns out to be dysplastic. Providing free access of offspring data to the public is a supplementary way to improve hip quality. The Swiss Bernese Mountain dog club has been publishing the offspring grades of each breeding sire since 1990, as soon as at least 10 offspring have been controlled (https://www.bernersennenhund.ch/chronik; accessed Sept 9, 2019). Offering this information allows breeders to exclude sires producing an excessive number of affected offspring from the breeding stock. Collection and publication of all data available, including those of lame elder dogs, should also be encouraged.

As CHD is a hereditary disease, several attempts have been made to localize the responsible genes (39). A genomic analysis should increase the accuracy of BVE and thus reduce the rate of CHD (40, 41). Compared to phenotypic selection, genomic selection has the advantage that the blood test is relatively cheap and can be done immediately after birth (42). This would allow breeders to keep valuable dogs in stock for later breeding. Currently genomic selection is not possible since the chromosome location of the genes determining hip conformation remains largely unknown (43). Hip conformation seems to be based on many genes with small effects, so that marker-assisted selection may not be successful either (44). A commercially available test for German shepherd dogs failed to show any positive effects and was considered unsuitable for CHD risk assessment (43). More research in different breeds is needed to establish genetic tests for early diagnosis and mass screening of puppies (42).

The overall scoring rate oscillated somewhat over the years. In Golden and Labrador retriever the rate was low (<15%) and dropped further. In Bernese Mountain dogs it was almost twice as high and increased slightly over the past few years. The Swiss Bernese Mountain Dog club established a health fund in 1999 to encourage its members to actively control the health status of their dogs (http://www.bernersennenhund.ch/club). This may

have helped to increase the scoring rate. The highest scoring rate was noted in the German shepherd dog. This may be associated with the common use of German Shepherds as working or sport dogs. However, whereas in German speaking countries i.e., Austria, Germany and Switzerland, all Retriever, Bernese Mountain and German shepherd dogs considered for breeding must be screened for CHD, in most other countries hip control is not mandatory and therefore only a small fraction of all breeding dogs is radiographed (26). In the UK for example selection of dogs used for breeding is left to the discretion of the owners with no restriction whatsoever (https://www.bva.co.uk/canine-health-schemes/hip-scheme/, accessed Sept 9, 2019). In the US no mandatory hip scoring is installed and the OFA registry is based on voluntary reporting (45, 46).

Some limitations of our results should be addressed. The scrutinizers in the two dysplasia committees varied over the entire study period although more than three quarters of all dogs were scored by the same four experts. Personal experience and new knowledge acquired over the years may also have influenced the scoring mode (47). The key limitation in most studies including the present one is the selection bias of the raw data (37, 45, 46, 48). Radiographs of dogs with clinical or obvious radiographic signs of CHD are less commonly submitted for official evaluation (45). This leads to an underrating of the true CHD-prevalence in the population. The true CHD-prevalence can therefore not be determined by scoring potential breeding stock only. As a consequence the Swiss Bernese Mountain Club changed their regulation in 2011 and decided that from every litter a particular number of random chosen dogs has to be evaluated for CHD (https://www.bernersennenhund.ch/reglemente-statuten, accessed Sept 9, 2019). Lack of data on dogs that were treated or euthanized because of CHD during the first year of life also is a strong bias. Lastly, radiographic evidence of hip osteoarthritis takes time to develop, and screening at 1 year of age based on the hip-extended view inevitably misses later onset and subtle radiographic signs of CHD.

In conclusion, the present study confirms that the prevalence of CHD could be reduced efficiently in five common large breeds in Switzerland over the last two decades using a systematic and strict phenotypic scoring scheme. However, the true prevalence of CHD is probably higher than reported. To put more downward pressure on the incidence and prevalence of CHD, additional programs should be considered such as breeding only dogs that are screened by a veterinarian and are publicly available, intermediate phenotype screening (measurement of joint laxity) and EBVs.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

AUTHOR CONTRIBUTIONS

All the authors contributed to the conception or design of the work, drafting and revising, and final approval of the version to be published.

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Heritability of Unilateral Elbow Dysplasia in the Dog: A Retrospective Study of Sire and Dam Influence

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Canine elbow dysplasia is a significant health issue affecting many breeds. Unfortunately, treatments remain relatively limited, so control of this disease often falls to selectively breeding for dogs with normal elbows. The objectives of this study were to evaluate the heritability of left-sided vs. right-sided elbow dysplasia, and to assess potential differential sire and the dam influence on offspring elbow status. In a retrospective study, elbow data from 130,117 dogs over 2 years old representing 17 breeds were obtained from the database of the Orthopedic Foundation for Animals and included in the study. Heritability estimates for unilateral elbow dysplasia varied between breeds (ranging from 0.01 to 0.36) and were similar between the left and right elbows. The estimated genetic correlation between disease in the left and right elbow ∼1 in the majority of breeds, with the exception of the hybrids, Australian Shepherds, and the Australian Cattle Dogs, likely due to low numbers of affected individuals. The sire and dam had equal impact on the offspring's elbow status. Furthermore, there was no increased risk for the sire or dam to pass on the same unilaterality of their elbow dysplasia to their offspring. However, the overall risk of elbow dysplasia in the offspring did increase when one or both parents were affected, though this also varied based on breed. Understanding of the impact that the sire and dam have on the offspring and of the overall heritability of both bilateral and unilateral elbow dysplasia is important in guiding breeding decisions to reduce the incidence in future generations of dogs.

Keywords: elbow dysplasia, heritability, dog, sire effect, breeds

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INTRODUCTION

A prevalent health issue that affects many breeds, particularly young medium to large sized dogs, is that of elbow dysplasia (1). Elbow dysplasia refers to the abnormal development of the elbow joint, resulting in early development of osteoarthritis and degenerative changes. Complex primary conditions associated with elbow dysplasia include fragmented medial coronoid (FCP), ununited anconeal process (UAP), osteochondrosis dessecans (OCD), and joint incongruency (1). The significance of having a dog diagnosed with elbow dysplasia is that treatments are not curative, and long-term prognosis is often poor. Surgery may be recommended for certain cases (2), but at this time the osteoarthritis and degenerative changes can only be treated with nutritional and medical management (3, 4), including maintaining a lean body weight, regular slow steady exercise as tolerated, physical rehabilitation, joint supplements, intra-articular injections, or oral medications to improve comfort (5).

Elbow dysplasia is an inherited disease, although it has been suggested that diet and exercise may influence the severity of the disease (1, 3, 6). Given that elbow dysplasia cannot be prevented in genetically predisposed dogs, and treatment is generally difficult, the primary method of controlling this disease is by attempting to eliminate elbow dysplasia through selective breeding. Several organizations throughout the world identify and record elbow dysplasia in dogs based upon radiographic evaluation and thereby provide tools to enable breeders to make informed decisions about which dogs to retain in their breeding programs. All organizations use screening protocols that comply with guidelines recommended by the International Elbow Working Group (IEWG) which grades elbows as normal (grade 0) or dysplastic, which then ranges from grade I to grade III dysplasia based on the severity of the degenerative changes. Importantly, general screening for elbow dysplasia only determines the phenotype of that particular individual dog, without predicting that dog's genetic makeup or its ability to produce unaffected puppies. Identifying the dog's phenotype significantly impacts the overall breeding value of that animal and improves the probability that the progeny also have improved phenotypes (7).

Environmental or physiological factors may affect the grading. For instance, a 1997 study by Corley et al., demonstrated that the Orthopedic Foundation for Animals (OFA) ratings on hips are increasingly more reliable as the dog reaches 2 years of age (8). Specifically, an assessment of normal hips for a dog between 13 and 18 months of age was 95% accurate when compared to the assessment of that dog at or over 2 years of age, leading to the determination that evaluations prior to 2 years of age are "preliminary" (8). Analogous data have not yet been studied in relation to the accuracy of diagnosing elbow dysplasia at early ages, but is suspected to be similar, especially given that previous data predicted only a slight influence of age on the presence of elbow dysplasia in dogs over 2 years of age (9). Some reports also indicate that male dogs are more frequently affected with elbow dysplasia than female dogs in certain breeds, such as in Labrador Retrievers (10). This is suspected to be correlated to hormonal differences between the sexes and a faster growth rate in male dogs.

Although the OFA does not recommend breeding dogs with any abnormal elbow result, regardless of dysplasia grade, some organizations such as the Federation Cynologique Internationale (FCI) and the British Veterinary Association (BVA) allow the breeding of dogs that are diagnosed with grade I elbow dysplasia as long as breeders proceed with caution and full awareness of the dog's other characteristics. However, previous research has shown that the risk of elbow dysplasia approximately doubles when one parent has elbow dysplasia, increasing from roughly 10–23% averaged across all dogs, with breed variations not considered (11). The risk of puppies developing elbow dysplasia has been shown to increase with severity of the disease in the parents. This indicates that there is a moderately high component of heritability to elbow dysplasia (11, 12). Overall, reports of heritability values for elbow dysplasia have been shown to vary significantly based on breed and population size (9, 13).

Within certain breeds, particularly those demonstrated to have a higher heritability, sires contribute slightly more in improving hip conformation within the population than dams do (9). This may, in part, be due to the popular sire effect in which certain males are bred widely to multiple females, but this sire impact has not been studied with consideration to individual contributions to the offspring or with consideration to elbow dysplasia. One small study involving Labrador Retrievers suggests there may be a maternal effect on the radiographic progression of elbow osteoarthritis in the offspring, but an underlying genetic component has not been evaluated (12) and the influence of the dam on elbow conformation includes factors beyond genetic contribution. The incidence of fragmented medial coronoid disease differs in prevalence between males and females, suggesting a sex-influenced component to inheritance (14). The presence of a strong maternal or paternal effect on offspring would impact breeder decisions on which dogs to continue using in breeding programs. Regardless of the genetic foundations of elbow disease, sire selection will always provide an outsized impact on breed improvement over that of dam selection, making the identification of superior sires all the more important.

It is also largely accepted that both hip and elbow dysplasia are most commonly bilateral, but that they may also present as unilateral disease. Anecdotally, many breeds appear to have a higher incidence of left-sided elbow dysplasia. Previous analyses using data published by the BVA (15) have shown that there is no difference in the heritability of right vs. left-sided hip dysplasia, but this has not been examined within elbow dysplasia. It is possible, therefore, that the heritability values may differ and may explain the anecdotal increase in prevalence of left-sided elbow dysplasia.

An objective of the present study was to evaluate whether differences existed between the heritability of dysplasia of the left and right elbows. An additional objective was to determine if the risk of elbow disease in offspring could be differentiated by the status of elbow disease in their sire vs. the elbow disease status in their dam. A final objective was to assess if a sire or dam with unilateral elbow dysplasia would pass on the same unilaterality of disease to their offspring, with the hypothesis that the same unilaterality would be inherited by the offspring with a higher frequency than the contralateral unilaterality. Characterizing the inheritance of elbow dysplasia will give breeders additional tools to reduce the incidence of this disease.

MATERIALS AND METHODS

Data collected from 1970 through November 2018 on 17 breeds of dogs older than 2 years of age were obtained retrospectively from the OFA database, and included dogs whose results were within the public domain and those whose results were withheld from public posting. If there were multiple submissions entered for a single dog, only the most recent submission was included for analysis. Only breeds having more than 380 elbow dysplasia submissions were used in the analyses.

Dogs were categorized as normal or dysplastic, and if dysplastic, they were sorted into bilateral disease, left-sided dysplasia, or right-sided dysplasia. There were no further classifications or distinctions made between the assigned grade of dysplasia or between any underlying pathology such as a fragmented medial coronoid, an OCD lesion, or an ununited anconeal process.

Data was then analyzed to evaluate the heritability of right-sided elbow dysplasia and left-sided elbow dysplasia and the relationship between them. Because it is feasible to treat the two elbow measures as two genetically distinct traits, the heritability of each elbow trait was estimated simultaneously as well as the genetic correlation between these measures. Anecdotally, investigators have suggested that when the genetic correlation between two traits exceeds 0.95 it is reasonable to consider the two traits as repeated measures of the same phenotype. The dataset permitted examination of that supposition.

Elbow dysplasia, on either lateral side, was measured as a binary characteristic (i.e., disease in the elbow is scored as 1, a normal elbow is scored as 0) and therefore, these two measures were approached as Bernoulli variables, considering the probability of disease in the j-th dog of the i-th sex as p_{ij}. This probability is commonly transformed to its log-odds, or logit ($\theta_{ij} = log(p_{ij}/(1 - p_{ij}))$, with the following representations for the left (L) and right (R) elbows:

$$\theta_{Rij} = \mu_R + \beta_R age_{ij} + sex_{Ri} + a_{Rij} + e_{Rij}$$
$$\theta_{Lij} = \mu_L + \beta_L age_{ij} + sex_{Li} + a_{Lij} + e_{Lij}$$

where $\mu_R$is an effect common to all dogs of a given breed, β_R is a regression coefficient accounting for the impact of age at screening to the risk of elbow disease, $sex_{Ri}$is the contribution of the i-th sex (i = M, F) to the risk of elbow disease, a_{Rij} is the additive genetic contribution to elbow disease for the j-th dog of the i-th sex and e_{Rij} is an unknown residual impacting the risk to elbow disease for the j-th dog of the i-th sex. Naturally, the subscript "R" defines those terms impacting disease of the right elbow, and those with the subscript "L" identifying the concomitant terms for the left elbow. To provide for the potential genetic correlation between elbow traits, we assume

$$\begin{bmatrix} a_R \\ a_L \end{bmatrix} \sim N\left[\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} A\sigma^2_{gR} & A\sigma_{gRL} \\ A\sigma_{gRL} & A\sigma^2_{gL} \end{bmatrix}\right]$$

and

$$\begin{bmatrix} e_R \\ e_L \end{bmatrix} \sim N\left[\begin{bmatrix} 0 \\ 0 \end{bmatrix}, \begin{bmatrix} I & 0 \\ 0 & I \end{bmatrix}\right]$$

where a_R (a_L)is a vector of additive genetic (breeding) values associated with disease of the right (left) elbow of all the dogs represented in the database for a given breed, A is the numerator relationship matrix constructed from the list of sires and dams extracted for each breed, σ^2_{gR} (σ^2_{gL}) is the additive genetic variance for the right (left) elbow trait, and σ_{gRL} is the additive genetic covariance for the right and left elbow traits. Finally e_R and e_L are vectors representing the unknown residual values for the right and left elbow traits, respectively, parameters that are set to have null means, unit variances and no covariance (16). Of course, it is then straightforward to extract the heritability of each lateral trait and their genetic correlation on this logit scale as

$$h^2_R = \frac{\sigma^2_{gR}}{\sigma^2_{gR} + 1}$$
$$h^2_L = \frac{\sigma^2_{gL}}{\sigma^2_{gL} + 1}$$

and

$$r_g = \frac{\sigma_{gRL}}{\sqrt{\sigma^2_{gR}\sigma^2_{gL}}} \quad .$$

Software that can accommodate the evaluation of correlated binary traits when there are underlying relationships among the recorded animals is not widely available. Fortunately, the publicly available package MCMCglmm (17) is readily adapted to this challenge using the public domain statistical platform R (18). The framework of this R package is Bayesian analysis, an approach that fits naturally with the outline of the model above.

As part of the Bayesian framework, the prior distributions for the putative fixed effects (i.e., constants, sex, and age effects) were noted to have independent normal densities with null means and variances of 10^{10}; that is, a diffuse normal prior. The prior distribution for the unknown covariance structure was assumed to be an inverse-Wishart density, provided by the package with parameters V, the scale matrix, and n, the degrees of freedom. Values for the genetic covariance matrix, which we set at the outset, were for V as an identity matrix (of order 2) and $n = 3$, a value which represents a flat prior across the interval [−1, 1] for the genetic correlation (17). The residual variance structure was fixed, as outlined in the model above, with the identity matrix of order 2 (16).

Estimates of the unknown parameters (i.e., sex effects, age effects, additive genetic values, variances, and covariances), and their transformation to heritabilities and the genetic correlation, are arrived at through a Markov Chain Monte Carlo (MCMC) process. After a series of preliminary analyses, we based our parameter estimates on a sample of 1,500 values from a single chain for each breed. The total number of samples generated was 200,000 with a "warm-up" period of 50,000 and a thinning rate of every 100-th sample [i.e., 1,500 = (200,000 – 50,000)/100]. The resulting chain was examined visually through trace and density plots for consistency, and autocorrelations were evaluated to insure that consecutive samples had a correlation <0.03 with the R package coda (19).

In addition to the estimation of these unknown genetic parameters, the number of matings between affected and unaffected males and females represented in this multi-breed database were counted. That is, all dogs were counted into one of four mutually exclusive categories: normal elbows, affected in left elbow only, affected in right elbow only and affected in both elbows. Similarly, when known, the sire and dam of each dog was counted into one of the same four mutually exclusive

TABLE 1 | Breeds analyzed, total number of dogs submitted for evaluation, categorization of the population size, and prevalence of any recorded elbow dysplasia within these dogs, presented alphabetically by breed name.

Breed	Breed code	Total number	Population size category	Elbow dysplasia prevalence
Australian Cattle dog	ACD	685	Small	0.085
Australian Shepherd	AS	3,346	Medium	0.027
Bernese Mountain Dogs	BMD	10,178	Large	0.234
Bouvier Des Flanders	BF	2,057	Medium	0.089
Bullmastiff	BMF	1,214	Small	0.145
Chow Chow	CC	381	Small	0.472
English Setter	ES	1,887	Small	0.144
English Springer Spaniel	EN	1,048	Small	0.159
German Shepherd dog	GD	13,243	Large	0.165
Golden Retriever	GR	26,401	Large	0.087
Greater Swiss Mountain dog	SMD	2,328	Medium	0.088
Hybrid	HY	522	Small	0.019
Labrador Retriever	LR	46,514	Large	0.091
Mastiff	MF	3,982	Medium	0.128
Newfoundland	NF	4,146	Medium	0.214
Rhodesian Ridgeback	RR	5,005	Medium	0.050
Rottweiler	RO	7,180	Medium	0.345

TABLE 2 | Heritability estimates + standard deviation of the MCMC sample of left and right elbow dysplasia by breed, shown alphabetically by breed code.

Breed	Heritability of elbow dysplasia in the left elbow	Heritability of elbow dysplasia in the right elbow
ACD	$0.08 + 4.6 \times 10^{-3}$	$0.06 + 5.7 \times 10^{-3}$
AS	$0.02 + 6.9 \times 10^{-4}$	$0.01 + 8.6 \times 10^{-4}$
BF	$0.06 + 1.8 \times 10^{-3}$	$0.11 + 1.1 \times 10^{-3}$
BMD	$0.21 + 5.0 \times 10^{-4}$	$0.22 + 4.0 \times 10^{-4}$
BMF	$0.08 + 2.6 \times 10^{-3}$	$0.11 + 2.6 \times 10^{-3}$
CC	$0.28 + 8.7 \times 10^{-3}$	$0.29 + 8.2 \times 10^{-3}$
EN	$0.28 + 3.1 \times 10^{-3}$	$0.19 + 3.4 \times 10^{-3}$
ES	$0.36 + 2.1 \times 10^{-3}$	$0.32 + 2.1 \times 10^{-3}$
GR	$0.16 + 1.8 \times 10^{-4}$	$0.13 + 1.8 \times 10^{-4}$
GS	$0.20 + 3.5 \times 10^{-4}$	$0.20 + 3.5 \times 10^{-4}$
HY	$0.01 + 1.8 \times 10^{-3}$	$0.01 + 8.7 \times 10^{-4}$
LR	$0.16 + 9.3 \times 10^{-5}$	$0.14 + 9.3 \times 10^{-5}$
MF	$0.10 + 7.9 \times 10^{-4}$	$0.10 + 9.5 \times 10^{-4}$
NF	$0.20 + 9.3 \times 10^{-4}$	$0.21 + 7.8 \times 10^{-4}$
RO	$0.31 + 5.9 \times 10^{-4}$	$0.30 + 5.9 \times 10^{-4}$
RR	$0.14 + 7.1 \times 10^{-4}$	$0.10 + 8.5 \times 10^{-4}$
SMD	$0.25 + 2.1 \times 10^{-3}$	$0.22 + 1.7 \times 10^{-3}$

categories. With these counts, the frequency of each mating type along with the offspring outcomes from each mating type can be evaluated. Predictions of the probability for each of the four offspring phenotypic outcomes in each of the 16-possible mating types was facilitated with the *multinom* command, fitting a log-linear model to these nominal categories, available in the R package nnet (20).

RESULTS

A total of 130,117 dogs were included in the study, ranging from 24 to 190 months old. The mean age was 31.8 months old. **Table 1** lists the breeds included along with their respective breed acronym codes, number of dogs evaluated, and prevalence of any elbow dysplasia. The breeds were then aggregated based upon number of dogs: the small population included breeds with fewer than 2,000 dogs (ACD, BMF, CC, EN, ES, and HY), a medium population included 2,000–10,000 dogs (AS, BF, MF, NF, RR, RO, and SMD), and a large population included more than 10,000 dogs (BMD, GR, GS, and LR).

The heritability of unilateral elbow dysplasia, varied substantially with each breed as shown in **Table 2**. The lowest heritabilities were noted in Australian Cattle Dogs, Australian Shepherds, and Hybrids. The highest heritabilities for unilateral elbow dysplasia were observed in Chow Chows, English Setters, and Rottweilers.

There was no statistically significant correlation between population size and heritability value. The genetic correlation between left and right elbow dysplasia varied based on breed, but was close to 1 in the majority of breeds examined. The relationship in genetic correlation between right and left elbows for each breed is displayed in **Figure 1**. This indicates that the heritability estimates for the left and the right elbow were not significantly different. The range was from 0.13 in hybrid dogs to 0.99 in Bernese Mountain Dogs.

Displayed in **Figure 2** are the average probabilities of progeny to have either normal or dysplastic elbows for all possible sire and dam combinations, across the four possible offspring elbow phenotypes. These values were computed from the complete database, across all breeds, to visualize the possibility that sires and dams may have an unequal impact on progeny phenotypes.

Interestingly, there were also certain breeds for which there has been a continued high proportion of matings that included dysplastic dogs over the years. **Figure 3**, including only dogs with known elbow phenotypes, represents the trend over time of breeders' willingness to exclude dogs with elbow dysplasia from breeding programs in different breeds over time. The Chow Chow, for example, recorded an average of only 31.7% of the known mating pairs as involving two normal dogs throughout the years. That percentage further decreased when dogs of unknown or untested elbow status were included in the analyses. Only 5.3% of all total recorded breedings for the Chow Chow breed were between two normal dogs. A total of 45.5% of recorded breedings for Chow Chows were between two dogs with unknown elbow status. While the Chow Chows consisted of a small population within this study, similar findings were noted within the Rottweiler breed. Of those matings with known sire and dam elbow grades for Rottweilers, only 51.4% were between two normal dogs. When including dogs with unknown elbow

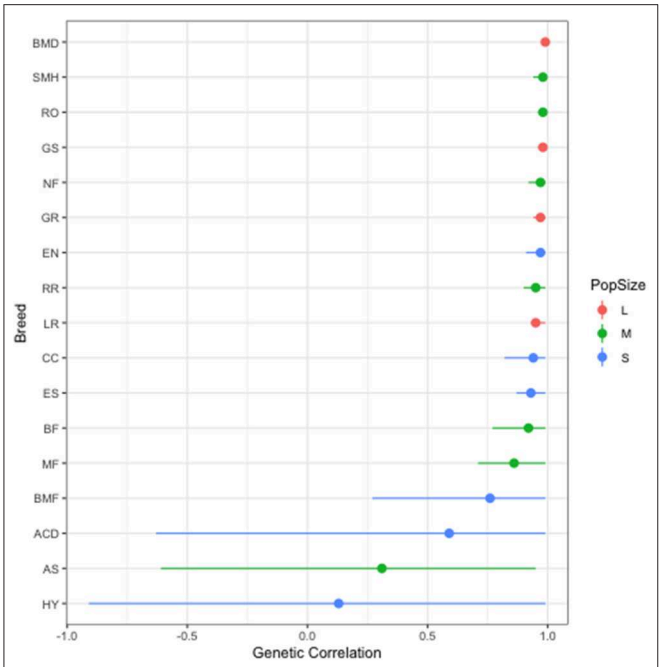

FIGURE 1 | Genetic Correlation between left and right elbow by breed. The colors reflect the population size category based upon the number of dogs of a given breed evaluated: large (L), medium (M), or small (S). Values are presented as mean (dot) and the 95% confidence interval (line).

phenotypes, recorded matings between two normal Rottweilers decreased to only 14.4% while 39.9% of all breedings were between two dogs with an unknown elbow status, and 3.1% of all breedings were between two dysplastic dogs.

In contrast, the Australian Shepherd breed has consistently retained only a low proportion of matings over the years involving dysplastic dogs, with no known breedings to any dogs with elbow dysplasia recorded in the most recent years. Rhodesian Ridgebacks have similar statistics, with 96% of the known, recorded breedings being between two normal dogs. If mating pairs with unknown elbow status were included in the calculations, 47% were determined to be between two normal dogs and 22% between two dogs of unknown status. This is a considerably higher proportion of normal-to-normal matings than what is observed in the breeds such as the Chow Chow or Rottweiler. Hybrid dogs were excluded from this analysis due to a low percentage of recorded parent data.

The prevalence of disease seen in the offspring of the different combinations of matings was also calculated and is presented in **Table 3**. While this is a less accurate method of evaluating the inheritance pattern of elbow dysplasia than by using heritability values, it does confirm and reflect the calculated heritabilities and the risks associated with breeding affected dogs. In Rottweilers, for example, the prevalence of disease seen when breeding two normal parents was 25.2%. This increased dramatically, to 41.6%, when one parent was affected and even more so when both parents were affected (56.1%). In Labrador Retrievers, normal parents were observed to produce affected offspring 8.3% of the time. The proportion of affected offspring increased to 16.1% when one parent was also dysplastic, and increased further to 30% when both parents were dysplastic. This is repeated in the majority of the breeds evaluated, with the exception of those whose affected population was low. Bullmastiffs, for example, only had one reported pairing of two dysplastic parents, and the single offspring screened for elbow dysplasia was reported as normal. Several breeds, such as the Australian Cattle Dogs, Australian Shepherds, and Bouvier de Flanders had no reported pairings of two dysplastic dogs, and therefore the prevalence of elbow dysplasia in offspring of these pairings were falsely reported as 0%. For Golden and Labrador Retrievers, the average estimated breeding values associated with elbow dysplasia exhibited a negative trend (**Figure 4**).providing evidence that these breed populations are improving elbow conformation, albeit slowly when compared to changes seen for hips in these same breeds (7).

DISCUSSION

Though there have been suggestions that there may be differences, the heritability estimates of left vs. right-sided elbow dysplasia were comparable as were the impacts of the sire or the dam on the elbow scores of the offspring. There was also no relationship between population size and heritability observed, though the heritability values varied significantly based on breed.

This study yielded either similar or slightly lower heritability estimates than have previously been reported for elbow dysplasia (9, 13). This is likely due to the heritability values being divided into left-sided and right-sided heritability rather than indicating bilateral disease or generally being categorized into elbow dysplasia. However, it is also possible that these values may have decreased, and may continue to decrease, as more breeding dogs are being health tested, and as selection pressure is applied in the majority of breed.

Some breeds, as demonstrated in **Tables 2**, **3**, have a much greater estimated heritability and prevalence of elbow dysplasia than others. The Chow Chow and Rottweiler, for example, have a much greater risk of producing affected puppies from normal parents than does the Australian Shepherd. This may be due to the overall genetics or conformation of the breed. However, it is also possible that this reflects the diligence of the breeders themselves. It is interesting to note that those breeds with higher heritability estimates (BMD, CC, ES, GS, NF, RO, and SMD) are the same breeds that have a continued higher prevalence of elbow dysplasia. These are also the breeds that have higher proportions of affected dogs included in breeding pairs, or those that have an increased number of pairings between dogs with unknown sire and/or dam elbow status. Chow Chows, for example, have a high proportion of breeding pairs that include a dog with elbow dysplasia. It is unclear if this trend is due to the poor compliance or culture within the Chow breeders, or if the proportion of affected dogs is so high among within this breed that eliminating all of these dogs from the breeding pool would be detrimental to the sustainability of the breed itself. Given the higher heritability values in these breeds, it is reasonable to conclude that the

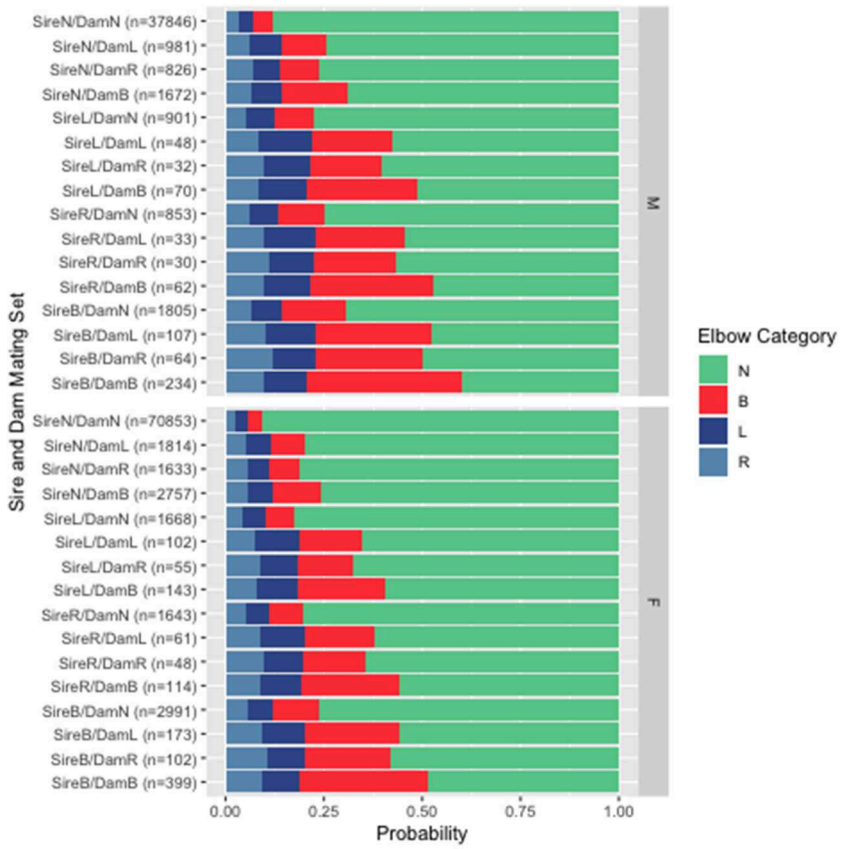

FIGURE 2 | Probability that male (M) and female (F) progeny will have normal (N), left (L), right (R), or bilateral (B) elbow dysplasia as a function of elbow status of sire and dam indicated by the breeding combinations on the left. *n*, number of breeding combinations in each category.

prevalence of elbow dysplasia could be decreased with more widespread screening of the parents and more careful selection of breeding normal dogs. Considering the number of affected dogs within these populations, strictly limiting breeding pairs to only unaffected dogs would severely limiting the genetic pool, which is undesirable. However, if breeders continue to breed affected dogs, even those with grade I elbow dysplasia, then the prevalence of elbow dysplasia within the breed is unlikely to decrease substantively and elbow dysplasia will continue to be an issue within these breeds. Additionally, for those breeds with a higher proportion of normal to normal matings, it is unclear if this was due to the overall low incidence of elbow dysplasia in these breeds, or if the low incidence and lower heritability values of elbow dysplasia are because of the continued compliance in breeding dogs with normal elbows. Rhodesian Ridgebacks similarly have had a historically low proportion of affected dogs being bred, and have a relatively low heritability value for elbow dysplasia.

Improvements in the incidence of both hip and elbow dysplasia over time have been noted, despite the screening process and compliance to selective breeding being entirely voluntary (9). The BVA has also reported similar findings within the population of dogs submitted for evaluation. The overall percentage of dogs in the UK with normal elbows has increased steadily from 70.0% in 1999 to 84.4% in 2016. The number of overall submissions has also increased dramatically from 583 in 1999 to 4,176 dogs in 2016 (21). The New Zealand Veterinary Association (NZVA) reported small but favorable genetic trends in reducing elbow dysplasia in German Shepherds, Rottweilers, and Golden Retrievers between 1992 and 2013 (13) as was seen in a Swedish population (22). Unfortunately, eliminating dogs with grade II or grade III elbow dysplasia from the breeding pool only excludes ∼4–8% of dogs, which does not exert a high selection pressure to rapidly improve elbow conformation (7, 15). This is in contrast to hip dysplasia statistics, where approximately 18% of dogs are eliminated from breeding programs due to inadequate hip formation (7). The improvement in elbow dysplasia has all been achieved by selecting phenotypically normal dogs for use in breeding. However, elbow dysplasia is a complex, multi-factorial disease, and even with two normal parents there is no guarantee for disease-free puppies. To overcome this limitation, estimated breeding values (EBV), based upon familial expression of the disease, can be used to improve the predictability that a given dog may pass on a disease/condition to its offspring. The EBV of a dog factors in the quality of both the individual dog's parents, their relatives and offspring produced, which is

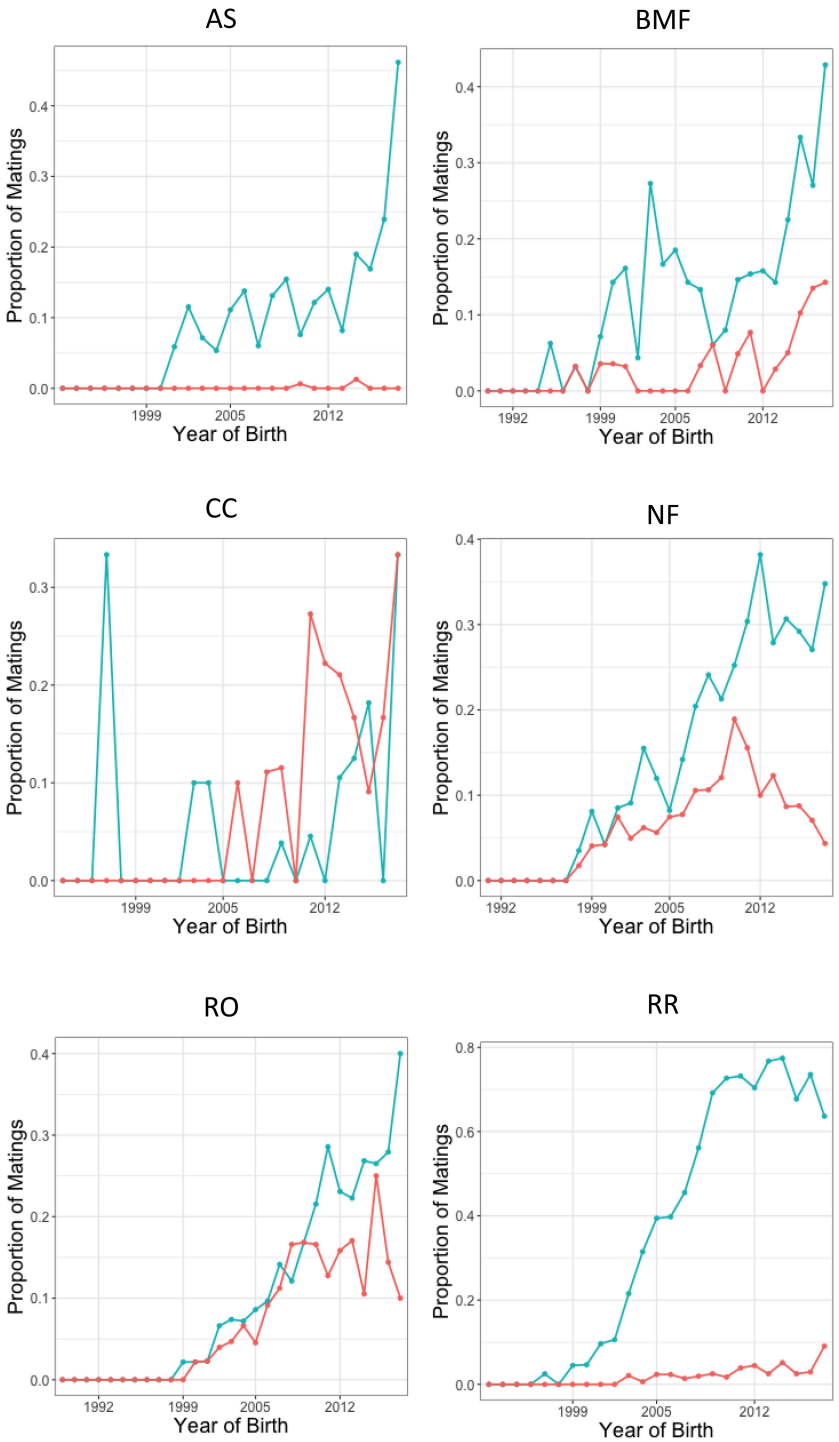

FIGURE 3 | Mating proportions of different breeds (AS, BMF, CC, NF, RO, and RR) over time, recorded at the time of offspring birth. The blue line represents recorded matings of normal-normal dogs. The orange line represents recorded matings of normal-affected dogs. Note, the axes' scales differ.

considered a more accurate representation of the dog's genetic quality than an individual record (23). EBVs may increase the rate of improvement in elbow or hip dysplasia within the population. However, EBVs are just starting to become more available in many countries through kennel clubs and are not yet commonly used among the majority of breeders in the USA (24). For the two breeds in this report with sufficient numbers of elbow evaluations to assess the genetic propensity contributing to elbow dysplasia, EBVs decreased over time and the genetic progress for Labrador retrievers was of a similar magnitude seen in that

TABLE 3 | Prevalence, by breed, of elbow dysplasia in offspring based on parent phenotype.

Breed	Prevalence of elbow dysplasia in the offspring, given parent combinations of:					
	Normal × normal	**Normal × affected**	**Affected × affected**	**Normal × unknown**	**Affected × unknown**	**Unknown × unknown**
ACD	0.071	0.222	–	0.078	0.375	0.072
AS	0.026	0.375	–	0.030	0.071	0.024
BF	0.082	0.171	–	0.090	0.077	0.089
BMD	0.184	0.304	0.389	0.230	0.329	0.246
BMF	0.179	0.143	–	0.140	0.179	0.134
CC	0.174	0.415	1.000	0.377	0.571	0.527
ES	0.111	0.289	0.778	0.112	0.329	0.140
EN	0.113	0.396	0.667	0.128	0.152	0.161
GR	0.072	0.159	0.400	0.083	0.196	0.095
GS	0.127	0.231	0.429	0.158	0.226	0.171
LR	0.083	0.161	0.30	0.085	0.152	0.097
MF	0.108	0.166	0.500	0.119	0.228	0.140
NF	0.198	0.264	0.484	0.189	0.324	0.206
RO	0.252	0.416	0.561	0.288	0.419	0.361
RR	0.050	0.075	–	0.042	0.108	0.055
SMD	0.057	0.178	0.533	0.115	0.206	0.097

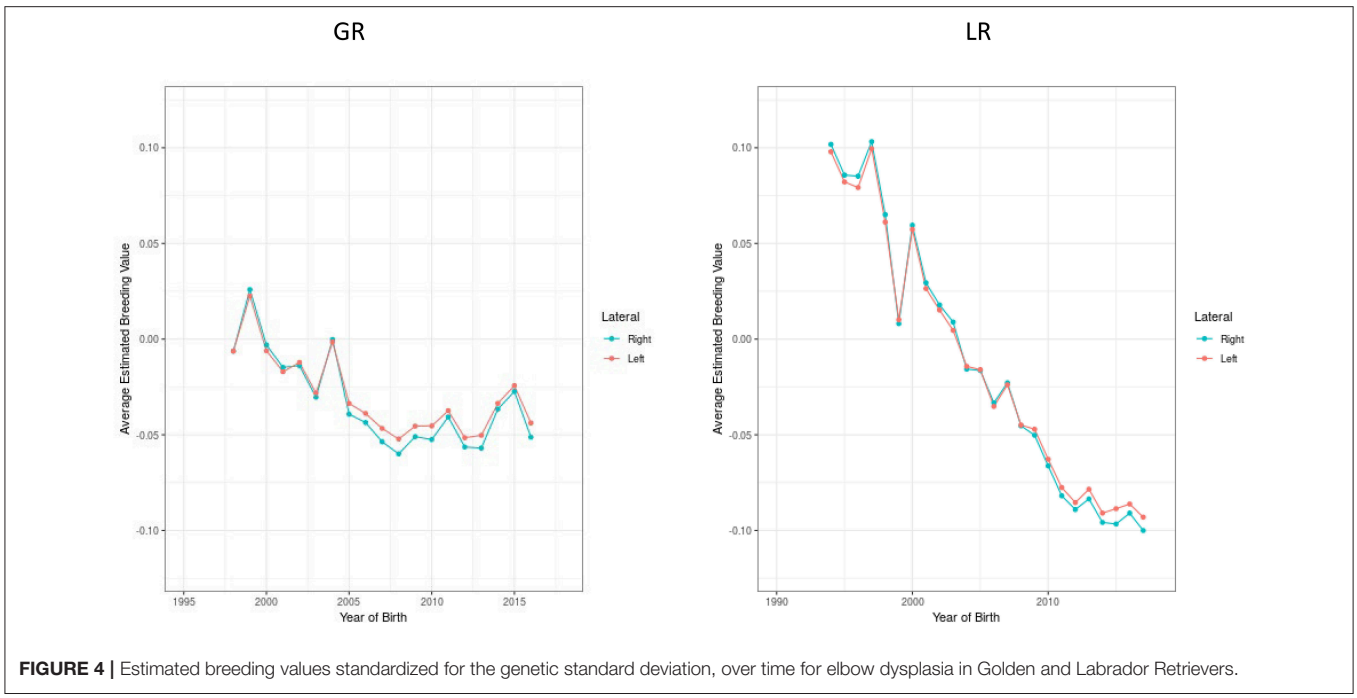

FIGURE 4 | Estimated breeding values standardized for the genetic standard deviation, over time for elbow dysplasia in Golden and Labrador Retrievers.

breed assessed in the United Kingdom (7). Implementation of more widespread diagnostics and/or EBVs could substantially reduce the prevalence of elbow dysplasia. This study confirmed the importance of ensuring both the sire and the dam have appropriate elbow clearances prior to breeding.

In this study, the calculations performed did not subdivide the dysplastic dogs into their respective grade of elbow dysplasia or primary disease process, largely out of concern for losing analytical power because of low numbers of affected dogs in each group. Therefore, the heritability values calculated were an average of all abnormal grades. It has previously been shown that the percentage of affected offspring increase with the severity of disease in the parent (25), so it is suspected that there may be additional genetic factors that influence inheritance or expression of these traits in the offspring of more severely affected dogs. This also may have precluded the detection of any maternal or paternal effects, as a maternal effect has been suggested in the inheritance of fragmented medial coronoid disease. The lack of a distinct maternal effect on the risk of the progeny inheriting elbow dysplasia within this study is in contrast to a previous study

(12). No overall maternal or paternal effects were observed in this study when grouping all abnormalities into elbow dysplasia. Any further analyses on individual subcategories would require a larger population of affected dogs for more accurate heritability values and further study of parental effects.

The low genetic correlation value seen between the left and right side estimates in the Hybrids (0.13), Australian Shepherds (0.31), and Australian Cattle Dogs (0.59) was most likely due to the smaller sample size and the limited number of affected dogs in these breeds. For example, out of 3,346 Australian Shepherds, only 53 elbows were graded as unilaterally dysplastic. Additional research with a larger affected population size would be necessary to determine if the findings were reflective of true differences within these breeds.

There were limitations to the study in that it was a retrospective study, and planned breedings between different elbow phenotypes with subsequent follow-up of all progeny were not done. As a retrospective study, there was no control for factors in image acquisition (such as increasing age or positioning) or for environmental factors such as the dog's activity or nutrition. Utilizing a large number of dogs with over a number of years and generations was done to counteract that those limitations. Also, it is likely that data used in the study were biased to a degree, as some owners will not submit radiographs if it is obvious that the dog is dysplastic. However, this study utilized the abnormal elbow grades that were not made available to the public, and therefore the bias was minimized. Furthermore, a substantial number of dogs were investigated in this study, and the trends remain consistent with those of previous studies.

Additionally, the diagnosis of elbow dysplasia is based upon the presence of one of several conditions (FCP, UAP, OCD, joint incongruity). These conditions reflect different developmental anomalies, but are all classified more generally as elbow dysplasia. The complexity of the multiple possible disease processes grouped together under the category of elbow dysplasia contributes to low heritabilities and slower genetic progress in reducing the overall incidence of elbow dysplasia. These separate disease complexes were not considered individually during this study.

The use of varying diagnostic methods to diagnose elbow dysplasia was not differentiated in this study, and the diagnosis of the final grade these dogs received was based on a minimum of a single flexed lateral radiograph of each elbow. The use of radiographs provides owners and breeders with a feasible and overall well-accepted method of phenotypic screening. However, it is widely accepted that computed tomography and arthroscopy are of higher diagnostic quality and are considered the gold standard for diagnosing elbow dysplasia (26, 27). This poses an ethical and political conflict, as computed tomography (CT) requires anesthesia and is more costly to the owners than is a radiographic exam, which may be taken awake or with varying degrees of sedation. The IEWG currently does not recognize a standardized method of obtaining CTs of the elbow to be used in the screening process, as joint congruency may still be affected by positioning and slice thickness of the CT used. Thus, in the absence of standardization, at this time the OFA, the FCI, and the BVA do not accept the use of CT to diagnose elbow dysplasia (26). With only one or two radiographic views required for screening, it is possible that some lesions may have been present but were not visible on radiographs (particularly only on a single lateral view). This would result in a small proportion of dogs rated as normal that perhaps should not have received a passing grade, and therefore are not eliminated from breeding programs. A standardized protocol for elbow CTs would be required in the future in order to use CT imaging as an alternative in health screening programs, particularly if the dog is borderline or questionable on elbow radiographs (27, 28).

In conclusion, as evidenced by the point estimates of heritability and the associated credible intervals that can be generated by the variability of these estimates, there were no substantive differences between the heritability of the left vs. right elbow, or of the sire vs. dam influence. Dogs with a particular unilaterality did not have a higher risk of passing down the same laterality to their offspring, although the risk of elbow dysplasia itself increased in the offspring when one or both parents were affected. While there have been some improvements in reducing the incidence of elbow dysplasia across many breeds since the initiation of phenotypic screening tools, the progression is significantly slower than the improvements seen in other diseases. Employment of widespread screening, judicious use of dogs in breeding programs, and the development and incorporation of EBVs may accelerate improvement. Elbow dysplasia can be a significant health concern for the affected dog and treatments are largely ineffective, which leads to control of this disease being based on selectively breeding for normal dogs. Therefore, control of the disease falls to the breeder's responsibility to have dogs tested and to make appropriate decisions on the suitability of their individual dogs for breeding in order to promote healthier generations of puppies.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for the animal study because the manuscript assessed only owner-submitted data without identifiers. No animals were directly involved with the study, only data. Written informed consent for participation was not obtained from the owners because no consent was necessary as the owners freely submitted the data to the health registry knowing that the data can be used for evaluative purposes.

AUTHOR CONTRIBUTIONS

Experimental design by GB, GK, and AO. Data analyses by GB, TF, and AO. Manuscript drafting by GB and AO with editing by all authors.

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Assessment of Image Quality in Digital Radiographs Submitted for Hip Dysplasia Screening

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Digital radiography is widely seen to be forgiving of poor exposure technique and to provide consistent high quality diagnostic images. Optimal quality images are however not universal; sub-optimal images are encountered. Evaluators on hip dysplasia schemes encounter images from multiple practices produced on equipment from multiple manufacturers. For images submitted to the Danish Kennel Club for hip dysplasia screening, a range of quality is seen and the evaluators are of the impression that variations in image quality area associated with particular equipment. This study was undertaken to test the hypothesis that there is an association between image quality in digital radiography and the manufacturer of the detector equipment, and to demonstrate the applicability of visual grading analysis (VGA) for image quality evaluation in veterinary practice. Data from 16,360 digital images submitted to the Danish Kennel Club were used to generate the hypothesis that there is an association between detector manufacturer and image quality and to create groups for VGA. Image quality in a subset of 90 images randomly chosen from 6 manufacturers to represent high and low quality images, was characterized using VGA and the results used to test for an association between image quality and system manufacturer. The range of possible scores in the VGA was −2 to +2 (higher scores are better). The range of the VGA scores for the images in the low image quality group (n = 45) was −1.73 to +0.67, (median −1.2). Images in the high image quality group (n = 44) ranged from −1.52 to +0.53, (median −0.53). This difference was statistically significant ($p < 0.001$). The study shows an association between VGA scores of image quality and detector manufacturer. Possible causes may be that imaging hardware and/or software are not equal in terms of quality, that the level of support sought and given differs between systems, or a combination of the two. Clinicians purchasing equipment should be mindful that image quality can differ across systems. VGA is practical for veterinarians to compare image quality between systems or within a system over time.

Keywords: visual grading analysis, digital radiography, PCA, image quality, hip dysplasia scheme

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1. INTRODUCTION

The major benefits of digital radiography are well accepted and include an increased tolerance of errors in exposure factor selection, avoidance of the negative environmental impact of film processing, ease of storage and retrieval, and if used correctly, improved image quality (1). For these reasons computed radiography or increasingly digital radiography dominate over film screen radiography in veterinary practice (2–4). This trend of digital radiography dominance is also seen in images submitted to the Danish Kennel Club for hip dysplasia screening. Over time, evaluators in this scheme have been under the impression that general image quality was related to the detector system manufacturer, which is known from the image metadata as the images are submitted in DICOM format. General quality is recorded by the screening evaluators for each image in the Danish Kennel Club database. This quality score is different to the hip evaluation score. The quality score is provided as a service to the veterinarians submitting images. It is based on a wide assessment including not only technical image quality issues, but also radiography technique such as patient positioning or beam centering. As such, this quality evaluation is not standardized and is not tested or further evaluated in this study. The evaluation was solely used to generate the hypothesis of a link between quality and detector manufacturer.

There is a perception that digital sensors and image processing and correct for all radiography errors, but in truth errors and artifacts also occur in digital systems (5). In the veterinary radiography literature there is lack of information on radiography quality control procedures specific to Digital radiography which are suitable for use in general practice. Quality control studies from the veterinary domain are limited in the literature. This sporadic occurrence of publications in this area suggests that there is poor awareness of the need to monitor image quality, both for reasons of diagnostic sensitivity and for maintaining radiation doses (to patients and, if present, technicians or owners) as low as reasonably practicable. A need for ongoing quality control in digital radiography is recognized in the human literature and a recent publication in the veterinary domain described work toward developing a quality control test specimen that may be pertinent to veterinarians (6). Procedures to achieve this may include rejected image analysis, exposure analysis, and artifact identification. All are suggested as being vital for the optimal operation of a department performing digital radiography (7). Reject image analysis in this context will include an assessment of image quality.

Digital image quality can be characterized by several parameters. Important among these are resolution, noise and artifact (8). Resolution describes the ability of the imaging system to separate features in the patient that are close to each other. These features may be close in the sense of physical space, in which case the term spatial resolution is used. This indicates the ability of the imaging system to display closely positioned features at separate locations. Alternatively two features may be close to each other in that they differ slightly in their ability to attenuate x-rays, in which case the term contrast resolution is used. It indicates the ability of the imaging system to display these similar but differently attenuating features at different points on a gray scale. An imaging system that can combine good spatial and contrast resolution will allow the radiologist to identify small objects that differ only slightly in their attenuating properties with surrounding tissue. Veterinary patients are often small relative to those encountered in human radiography and so may be particularly demanding of good spatial resolution. System noise may be seen as variations in image pixel value that are unrelated to the attenuation properties of the tissue being imaged. If an area that is expected to show uniform attenuation (e.g., muscle), shows random variations in pixel value (gray tone), this may be due to system noise. The inverse relationship between noise and the number of photons used to obtain the radiograph is important in radiography. The term "anatomical noise" refers to the role that normal anatomy may have in obscuring important pathology. Anatomical noise is considered to be the limiting factor in the detection of lesions in the thorax (9, 10). Artifacts can be thought of as features that are seen in an image and mask or mimic clinical features. Digital image artifacts can arise within the patient, or within acquisition hardware or software.

Methodologies for quantifying these parameters of image quality may be physical measurement, psychophysical evaluation or clinical assessment. Physical measurements include detective quantum efficiency (DQE) methods which are concerned with parameters such as modulation transfer function (MTF) and noise power spectrum (NPS). DQE methods are objective but are considered indirect methods of image quality. Descriptions of image quality from these physical perspectives give information about technical image quality, without any influence of human observers. Psychophysical methodologies of image quality assessment include the "contrast detail" analysis. Observers are asked to score images from phantom objects and the results provide quantitative assessments of low contrast and small detail measurements. These measurements correlate well with performance measurements in chest radiography (11). Both the physical and psychophysical methodologies however are based on measurements from phantom objects and can be criticized for not reflecting realistic clinical image environments (12). Performing and interpreting objective physical measurements of image quality are likely beyond the veterinary practitioner who may be considering the purchase of new imaging equipment, or concerned with maintaining and improving image quality over time with existing equipment as part of a quality control procedure. Visual grading analysis (VGA) is a clinical assessment, and is accessible to the veterinary practitioner for image quality audits. It is based on the ability of observers to detect and perceive predefined image criteria (13). It is an image evaluation methodology that is reported to be have attractive simplicity and powerful discriminating properties (14, 15). A VGA may be performed using absolute or relative grading. In the former, assessors score the degree to which specific image criteria are met. Relative grading on the other hand compares specific image criteria in the image being assessed to the same criteria on a reference image. This latter form of VGA was used in this study and our 5 point grading scale is typical. In relative VGA, a high score simply indicates the degree to which the image examined is

better than the reference. Both the reference and test images may be excellent or both may be poor.

The aims of this study are to confirm the suspected association between quality grades in the kennel club records of hip dysplasia screening radiographs and the system manufacturer, and to rank manufacturers according to image quality. If the suspected association is confirmed, this ranking will be used to create two groups of manufacturer by image quality. Visual grading analysis will then be used to test the hypothesis that images chosen at random from each of the two image manufacturer groups differ with respect to image quality assessed by VGA. The null hypothesis being that there will be no difference in VGA quality scores between the two groups.

2. MATERIALS AND METHODS

Digital radiography images (16,360) submitted during the period 2012 to 2017, to the Danish Kennel Club for hip dysplasia screening were retrieved from a patient archiving and communication system (PACS). For each image the manufacturer name (available as metadata in the header of each image) was retrieved, as was a kennel club quality grade awarded at the time of hip dysplasia grading. The grade uses an ordinal scale with three categories 1, 2, and 3 and was stored in the kennel club database. A kennel club quality grade of "1" is applied to images of satisfactory quality and grades of "2" and "3" applied to images with increasing degrees of technical faults, but are nonetheless of diagnostic quality for the purposes of awarding a hip dysplasia screening score. Technical faults in this context include suboptimal image contrast and spatial resolution, the presence of noise and also artifacts unrelated to the detector system such as labeling and positioning errors. The images were ranked according to their kennel club image quality grade. Statistical analysis was performed on these data as part of the hypothesis generating process to confirm that an association exists in the database between manufacturer and quality grade and also to determine mean kennel club quality grade for each manufacturer. The quality grade was then used to create a list of manufacturers ranked by image quality. Images from the top three ranked manufacturers were assigned to a group (high quality), and images from the bottom three manufacturers were assigned to a group (low quality), for VGA analysis as described below. This study was carried out in accordance with the commitments contained in the Basel Declaration and adhered to the General Data Protection regulations of the European Union. The protocol was approved by the local Ethics and Administration Committee, Department of Veterinary Clinical Sciences, University of Copenhagen.

2.1. Visual Grading Analysis

The three manufacturers with the three highest average kennel club quality grades (high quality group) and the three with the three lowest grades (low quality group) were selected for the VGA. Fifteen images from each manufacturer were randomly selected for evaluation, resulting in a total of 45 images per group (total 90 images). The null hypothesis was that there would be no difference in VGA scores between groups. In addition three images from each manufacturer (i.e., 18 images) were duplicated. These duplicates were combined with the 90 images mentioned above and again presented randomly. Their scores were used for measuring intrarater agreement. Thus, 108 images in total were analyzed. All images were compared during the analysis to a "reference" image chosen at random from a set of images from the manufacturer with the median quality grade in the kennel club database. In this way the VGA used can be described as a "relative VGA."

Five VGA image criteria as follows were used. Criteria "A" and "C" were concerned with contrast resolution and low contrast resolution, respectively. Assessment of criterion "A" compared the demarcation between medullary and compact bone (mid diaphysis right femur) in the test image to that of the reference image. Criterion "C" compared the visualization of the acetabulum as it summates with the femoral head on the test image to that of the reference image. Criteria "B" and "E" were concerned with spatial resolution. Assessment of criterion "B" compared the sharpness of bone trabecula in the right femoral neck and greater trochanter area in the test image with that of the reference image. Assessment of criterion "E" compared the sharpness of the right femoral head on the test image with that of the reference image. Criterion "D" was concerned with image noise. Assessment of this criterion "D" compared the homogeneity of the soft tissues lateral to the mid diaphysis of the right femur with that of the reference image. These various image criteria were chosen to be relevant to the imaging task at hand, namely the evaluation of pelvis radiographs for hip dysplasia screening, and to correlate with those reported for similar imaging tasks in the literature (16). Scores were awarded on a 5 point scale, with scores of -2, -1, 0, $+1$, and $+2$ to indicate that a criterion is, respectively, much worse than, worse than, similar to, better than or much better than the same criterion on the reference image The mean of the 5 individual image criteria scores was determined for each image for each reader, and the mean of these reader scores was taken as the overall VGA score for each image. The score for each image is thus a mean of means. The minimum score an image could receive was -2, the maximum $+2$.

2.2. Viewing and Assessment

Three observers performed the assessment, a veterinary imaging resident, a veterinary radiologist and a human certified reporting radiographer at two different viewing locations (University of Copenhagen and Odense University). At both locations images were viewed on paired DICOM standard screens using DICOM display software (ViewDex V2.48) which has been used and described in observer performance studies in radiology (17, 18). This allowed the observer to view the test and the reference images side by side, to zoom, pan, alter window level and width for each image, and to enter the assessment for each parameter using a check box available on the side of the image. Responses are automatically logged in data files for the program. Images were presented in random order by the software; there was no opportunity to revisit images already scored. The observers could interrupt their session at any time and subsequently pick

up where they left off. Each observer completed the task in 2 to 3 sessions.

2.3. Data Processing and Statistical Analysis

Data was extracted from image files using the PyDicom package (Version 1.2., available at https://pydicom.github.io/pydicom/stable/index.html) in Python (Version 3.7.2. Python Software Foundation, http://www.python.org). A Kruskal-Wallis rank sum test was used to test for associations between image quality grades from the kennel club database and the manufacturers. Differences in VGA test scores between the two quality groups were tested with a Wilcoxon rank sum test. Results of the VGA were explored using principal component analysis (PCA). Intraclass correlation coefficients for each observer were calculated to estimate repeatability of the VGA. Kendall's coefficient of concordance (Kendell's W) was used to the measure the degree of association between the assessments made by the three evaluators. All statistical tests and procedures, and the plot generation were performed using the statistical programming environment R (R: A Language and Environment for Statistical Computing, version 3.5.1, 2018, https://www.r-project.org/).

3. RESULTS

3.1. Overview of the Dataset

A total of 40 different manufacturer names were identified in the dataset. Images from 9 manufacturers were represented by 20 or less images. These manufacturers and images were excluded from further consideration. Images where no quality grade was available in the kennel club database were also excluded from consideration. This process of elimination resulted in 15,859 of the original 16,360 images available for further analysis. Of these 15,859 images, 12,685 (80%) had image quality "Grade 1," 2157 (14%) had image quality "Grade 2" and 607 (4%) had image quality "Grade 3." The mean quality grades for these 31 manufacturers ranged from 1 to 2.13. There was a statistically significant association between kennel club quality grade and manufacturer ($p < 0.001$). The images were thus grouped by manufacturer and groups were then ranked according to the mean quality grade for the manufacturer. High and low quality groups were thus created as described in the methods and a VGA was performed.

3.2. Visual Grading Analysis

The range of the VGA scores for the images in the "low quality" group ($n = 45$) was -1.73 to $+0.67$, with a median value of -1.2. The corresponding values for images in the "high quality" group ($n = 44$) ranged from -1.52 to $+0.53$, with a median value -0.53. This difference was statistically significant ($p < 0.001$). The image numbers per group are not equal as one image had to be rejected from the assessment. It was an elbow joint image that was accidentally inserted into the wrong database group during initial upload to the kennel club PACS.

The scores for each image criterion are shown for each quality group in **Figure 1**. It can be seen that for all image criteria, images in the high quality group outperformed those in the low quality group. The PCA of the data shows that despite some overlap, there was a separation in images according to image quality group when the first and second principal components of the VGA data were plotted against each other. The loadings shown on the biplot (**Figure 2**) indicate that there was a positive correlation between the assessment of image criteria A, B, C, and E. These criteria are concerned with contrast resolution (criterion A), spatial resolution (criteria B and E), and low contrast resolution (criterion C). The assessment of criterion D (image noise) was not correlated to the other evaluation criteria. In the PCA, 83.1% of the variation in the data was explained by the first two principal components (PC1 and PC2). The reference image and examples of the test images are shown in **Figure 3**.

3.3. Intraclass Correlation and Kendall's W Coefficients

The ICC was determined for each readers assessment of duplicate images. The values of this test statistic for the three readers were 0.907, 0.921, and 0.948. Values above 0.9 are considered to indicate excellent agreement (19). The value of Kendall's W coefficient of concordance was 0.8 (zero indicates no agreement between raters; 1 indicates perfect agreement).

4. DISCUSSION

This study set out determine if a suspected association between image quality and image detector manufacturer existed in a large cohort of images submitted for hip dysplasia screening to the Danish Kennel Club. The results of the initial analysis of the metadata from the images and their associated quality assessment indicate that one or more manufacturers are over-represented in one or more of the kennel club image quality grades. This demonstrates that there is an association between these quality grades and the system manufacturer associated with the image. The VGA was used to determine if an assessment based on image quality only, using carefully selected image criteria will also demonstrate differences according to manufacturer.

The evaluation criteria used in this study relate to detector and image processing performance and are particularly relevant to the evaluation of skeletal disease. With regard to the reference image, it is important to note that it should not be thought of as an ideal image. An optimal reference image from the point of view of the VGA will rank midway in quality with the test set images; some images in the test set will be found inferior to the reference, others superior. In this way if the reference image is ideal, the full range of test scores will be utilized in the assessment. The degree to which a reference image proves to be optimal only becomes clear as the study progresses, and only after an analysis of the results has been performed.

Choice of image criteria for evaluation is important. In this study image criteria A and C indicate contrast resolution, B and E are related to spatial resolution and criterion D relates to system noise. All were chosen with skeletal assessment in mind. Other relevant image criteria can be envisaged for assessment of other tasks, e.g., thoracic and abdominal imaging. In this study expert participation from academic radiographers and experience of

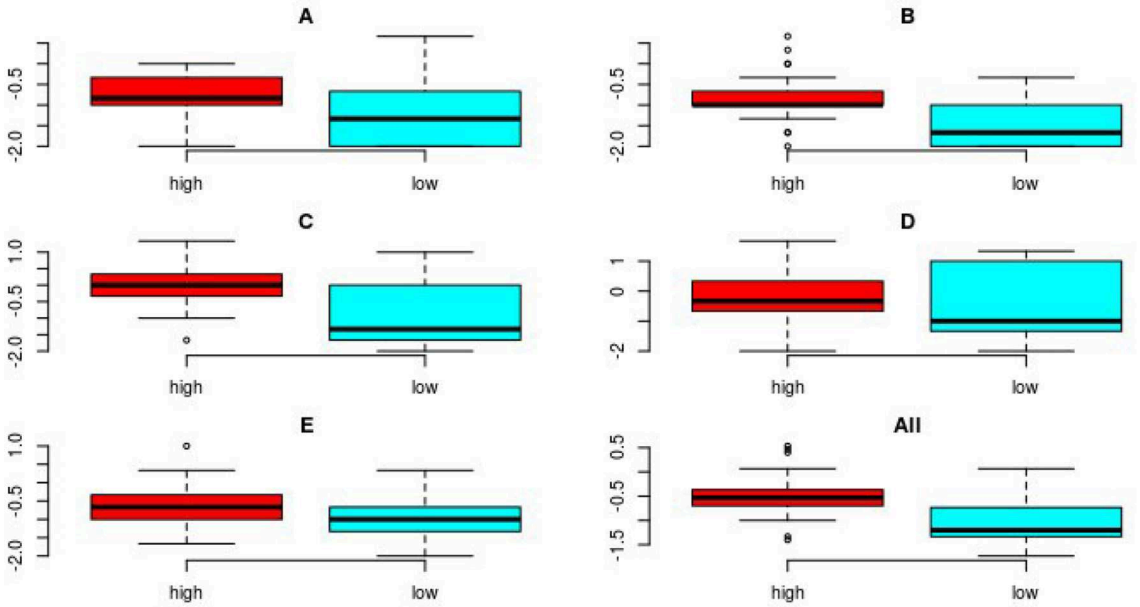

FIGURE 1 | Boxplots showing the combined results of the VGA for the three readers for all images for each of the 5 image criteria, **(A–E)** and for all criteria combined, for both image groups (n = 44 and 45 for high quality/low quality, respectively). The boxes show the interquartile range of the data, the whiskers are set at 1.5 times the interquartile range or the maximum value and minimum values if these values are smaller or greater, respectively. The horizontal line shows the median of the data. Possible outliers are plotted individually outside the range of the whiskers. The median value for each image criterion evaluated and for all imaging criteria combined, was higher (indicating superior image quality) for the images in the high quality group compared to median value for images in the low quality group.

veterinary imaging were combined to devise relevant image features for assessment by VGA. Well-chosen VGA criteria give rise to evaluations that are clinically relevant and allow an assessment process for the observers that is similar to their day to day clinical image evaluations.

The underlying technical influences on the outcome of the VGA have to be considered if system quality is found to be unsatisfactory. This consideration requires a record of the details of the image system and of the imaging parameters. This information is essential for remedial action. Details required will include focal spot size, degree of collimation, exposure tube current (mA) time (s), and kilovoltage (kVp), detector object distance, detector to focus distance, detector and anti-scatter grid specifications including fill factor, exposure index values, patient thickness, region examined and reconstruction algorithm used. If these parameters are known, a recommendation for image optimization can be made. Some of these data, specifically those relating to the x-ray generator and exposure factors, were not available to us in this study. Also the relative purchase costs of the equipment detector systems was not known. For this reason we cannot specify causes for the different image quality scores awarded. Of the list given here, many parameters will be constant within a practice, available in the system documentation or be self-evident from the image. It may be that only mA, s, kVp, patient thickness, exposure index values and collimation need to be recorded for each exposure by the radiographer for practical recommendations for improvement to be made.

The VGA results in this study show a significant difference in score between groups, indicating that veterinary imaging systems are not equal in terms of the image quality represented by their images in a large database. There are potential explanations for this. It may be that the hardware and software of one system are superior to those of another; it may be that systems are technically equal, but all do not run to manufacturer's specification either because qualified technical support is not available or not sought. There is some support for the latter view in that for most criteria shown in **Figure 1**, there is a greater variability in the VGA data for the low quality compared to the high quality group. The data therefore does not provide an ordered list of systems by quality to which manufacturers name can be fairly added. Such a list would require that comparisons were made between images of the same patient or object, a standardized technique and that all manufacturers confirmed that their systems were working and used according to their specification. The ranked list that such a study would produce could then be displayed along with equipment cost, or ranked lists could be grouped according to equipment cost. We feel, however, that given the number of images examined (almost 16,000 in the initial survey and 90 in the detailed VGA), bias that may be introduced by one patient type or other non-system variable is reduced. The high levels of agreement, with ICC values between 0.91 and 0.95, indicate excellent reliability for the VGA (20). Thus the data are a fair indicator of current status; an indicator that there is an association between image quality and manufacturer. It should also be noted that all the images included in the study were of diagnostic quality for the clinical indication at hand (hip dysplasia screening). However, other clinical scenarios can be envisaged where the shortfalls in spatial or contrast resolution or in image noise detected in the images examined could be limiting in diagnosis.

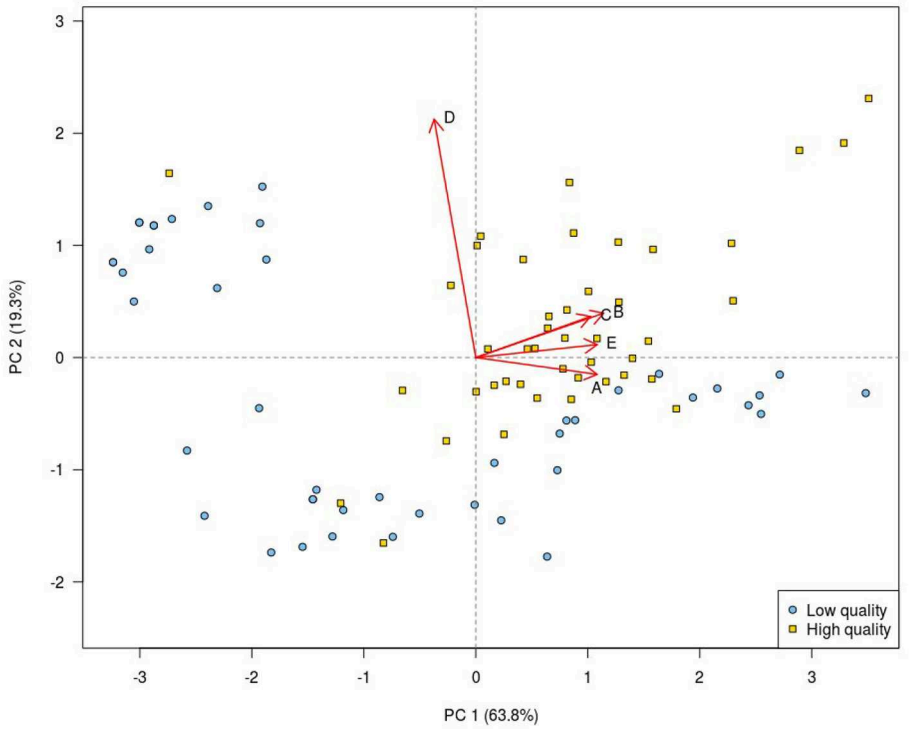

FIGURE 2 | Principal component analysis biplot of the VGA results. Each plotting character represents a single image. Principal component analysis scores for the high quality images (yellow squares) are grouped in the upper right quadrant, while PCA scores for the low quality images (blue circles) mainly occupy the remaining quadrants. This indicates that the VGA appears successful in separating the two image groups. The first (PC 1) and the second (PC 2) principal components taken together describe 83.1% of the variation in the data. The loadings (red arrows) show the contribution of each image criterion and their degree of correlation. The biplot shows that image criteria A, B, C, and E were positively correlated, while there was poor correlation between the PCA scores for image criterion D and those of all other image criteria.

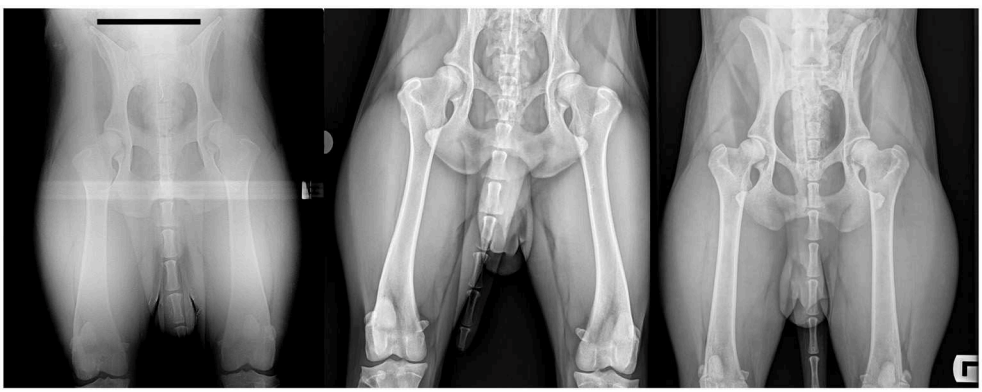

FIGURE 3 | Test images selected according to VGA results, and the reference image. The relatively low quality image (left) has an overall VGA value of −1.7. It thus lies on the spectrum between being worse (−1) and much worse (−2) than the reference image. The relatively high quality image (right) has an overall VGA value of +0.5. It thus lies on the spectrum between being similar to and better than, the reference image. The reference image is shown in the center. The assessment criteria called on the observers to focus attention on the region of the mid-diaphysis of the right femur and on the right hip/femoral trochanter region. Images have been cropped and identifying data masked for this figure.

A further study that eliminated variance in radiographic technique e.g., standard radiographic subject and consistent radiographer and technique might refine the findings. Those findings might then, quite reasonably, be correlated with the costs of the installation as well as the manufacturer. It is also quite likely that one manufacturer may produce installations of differing complexity, cost, and image quality; this would have to be considered.

Veterinarians should be aware of inequalities as demonstrated in this study, in image quality between systems from different manufacturers. Such awareness and a knowledge of image quality analysis, particularly of relative VGA, would allow practitioners

to make relevant quantitative image quality assessments as part of their purchasing, commissioning and quality control protocols. Expertise is available in the human radiography community and greater collaboration between veterinarians and this community would likely improve the general standard of quality control in veterinary imaging.

DATA AVAILABILITY STATEMENT

Anonymised datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The protocol was approved by the local Ethics and Administration Committee, Department of Veterinary Clinical Sciences, University of Copenhagen.

AUTHOR CONTRIBUTIONS

LM, HP, FM, and ES contributed to the conception and design of the study. HFP and FM organized the database, JJ, LM, and FM performed the VGA. HP, LM, JJ, and FM wrote the manuscript. FM performed the statistical analysis. ES and DN performed the initial image evaluations. All authors contributed to manuscript revision, read and approved the submitted version.

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Effectiveness of Canine Hip Dysplasia and Elbow Dysplasia Improvement Programs in Six UK Pedigree Breeds

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Hip and elbow dysplasia are common disorders in larger dog breeds and crosses, and a known contributory factor to osteoarthritis, lameness and reduced mobility. Screening schemes evaluating the severity of hip and elbow dysplasia in the UK are administered by the British Veterinary Association (BVA) and the Kennel Club (KC). The BVA/KC Hip Dysplasia scoring scheme is over 50 years old, having originated in 1965, and has operated in its current form since 1983. The BVA/KC Elbow Dysplasia grading scheme commenced more recently in 1998 and is based on the International Elbow Working Group guidelines. Hip score and elbow grade data on a considerable number of dogs in the UK have been generated from these two screening schemes. This study analyses data from dogs of six breeds scored from 1990 to present, to establish any determinable trends in hip score and elbow grade parameters, and to examine whether the implementation of such schemes has had a positive influence on hip and elbow health. A range of criteria, including the rate of participation in the screening schemes, hip score and elbow grade parameters (e.g., median, mean, standard deviation), and estimated breeding values (EBVs) were analyzed, both in the overall population and also among breeding animals. The results show a general decline in hip score parameters (median, mean, standard deviation, and 75th percentile), revealing a reduction in the prevalence and severity of hip dysplasia. There was a more modest decline in mean elbow grade within breeds. The proportion of sires and dams (of dogs born per year) with no hip score or elbow grade fell substantially over time, demonstrating good participation in the screening schemes. In most breeds, the scores of sires and dams are demonstrably improving. There is a declining genetic trend as ascertained by EBVs for both hip scores and elbow grades in most breeds, implying that the improvement observed is due in part to selection for improvement in hip and elbow health as described by the respective screening schemes.

Keywords: hip dysplasia, elbow dysplasia, canine health, selection, phenotypic improvement

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INTRODUCTION

Hip and elbow dysplasia are considered important hereditary orthopedic diseases that are known to be prevalent across several, in particular medium to large, dog breeds and their crosses, and have long been widely acknowledged to negatively impact the health and welfare of an affected individual (1). Hip and elbow dysplasia are categorized as developmental disorders caused

by dysmorphic and lax joint formation. This malformation consequentially results in abnormal wearing of bone over time, inducing the secondary development of osteoarthritis (OA) or arthrosis, and degenerative joint disease (DJD) (2). Elbow dysplasia can be categorized into four subsets of disease: osteoschondrosis of the medial part of the humeral condyle, fragmented medial coronoid process (FCP), ununited anconeal process (UAP), and incongruity of the elbow joint (3, 4). Unfortunately, the pathology of neither hip nor elbow dysplasia can be reversed and so, for an affected individual, the best outcome is management of the disease through pain medication or replacement surgery, with the latter having additional consequences of cost and an extensive recovery period (5). The underlying etiology of dysplastic disease is complex with a long understood knowledge of a genetic influence (6–8) and multiple environmental factors, such as obesity or over-exercise during growth (9–11). Given genetic influences contribute to an individual's risk of development of both hip and elbow dysplasia, the importance of selecting breeding stock with the aim of reducing genetic risk in future generations is seen as the most useful means to elicit a widespread and permanent reduction in disease (2, 12).

While there is an established underlying complex (i.e., non-Mendelian) genetic influence on dysplastic disease, the consequential lameness and DJD does not usually become clinically apparent until after breeding age. Therefore, screening schemes such as the British Veterinary Association (BVA)/Kennel Club (KC) Hip and Elbow Dysplasia Schemes have been established to give an indication of the severity of pre-clinical affectation, and so provide breeders with the ability to make informed decisions regarding which animals to use for breeding. Due to earlier recognition of hip dysplasia as a serious welfare impairment, the BVA/KC Hip Dysplasia scheme was launched in its current format in 1983, with the Elbow Dysplasia scheme following later in 1998, which follows guidelines provided by the International Elbow Working Group (IEWG). Scoring of an individual involves a ventrodorsal and mediolateral radiograph (for hips and elbows, respectively), before submitting to the BVA for scrutiny by a panel of veterinary experts in orthopedics and radiography. The criteria for hip radiograph scrutiny incorporate nine distinct features on each hip, each scored according to the degree of laxity and/OA with a final score established from the sum of the total for the left and right hip (13). With regard to elbows, the margins between the joint and the bone structures are measured, as well as signs of any primary lesions (an area of damage caused directly by disease) and/or OA (14). The minimum score is 0 and the maximum possible is 3, whereby the highest individual elbow score taken is the overall grade (e.g., a right elbow grade of 0 and a left elbow grade of 1 would be reported as grade 1).

For breeds where a significant proportion of the population has participated in dysplasia screening schemes, numerous studies have determined the heritability of various measures of hip dysplasia and elbow dysplasia, i.e., quantifying the extent of additive genetic variation underlying apparent phenotypic variation, across a variety of breeds and countries (12, 15–25). The moderate magnitude of the various estimates of heritability demonstrate that selection for improvement will be successful. Furthermore, in several countries individual estimates of genetic risk, estimated breeding values (EBVs), for evaluations of hip dysplasia (HD) and elbow dysplasia (ED) are routinely provided on registered pedigree dogs. EBVs provide a more accurate metric for selection than phenotypic measures of HD and ED since non-additive genetic (including environmental) effects are discounted and information on relatives (who share genetics) is taken into account. While several different loci having been identified as associated with disease (2, 26–28), it is not always clear what proportion of the additive genetic variance they comprise and it is likely that only genomic breeding values (gEBVs) will offer an effective "DNA test" for dysplastic disorders (29, 30). However, these will, like EBVs, take the form of a quantification of risk, rather than denoting binary categories of "affected" and "unaffected."

This study utilized screening data of UK Kennel Club registered dogs from six breeds born from 1990 to 2018 to establish any determinable trends in parameters, and to examine whether the implementation of such schemes has had a beneficial influence on overall hip and elbow health. A range of criteria, including the rate of participation in scoring schemes, score parameters (such as median, mean, standard deviation of scores, and grades), and EBVs were analyzed, both in the overall population and also among breeding animals.

METHODS

Data

Six breeds with EBVs for hip score and elbow grade from the BVA/KC screening schemes were included in the study: Labrador Retriever (LR), Golden Retriever (GR), German Shepherd Dog (GSD), Rottweiler (ROTT), Bernese Mountain Dog (BMD), and Newfoundland (NEWF). Participation in the BVA/KC hip and elbow screening schemes is voluntary and details of scoring protocols are given by Fluckiger (14) and Gibbs (31), respectively. In brief, radiographs of hips are scored bilaterally on 9 features according to the degree of laxity and/or OA observed. Eight features are scored from 0 to 6, and one feature is scored from 0 to 5, zero indicating an absence of, and higher numbers the severity of, pathology. The maximum score, indicating the most severe pathology, for each hip is 53. Both the individual totals for left and right hip are publically reported, along with the bilateral total score which ranges from 0 (indicating no malformation) to 106 (severe hip dysplasia). The BVA/KC elbow scoring scheme was launched in 1998 based on guidelines of the International Elbow Working Group (IEWG). Elbow radiographs are scored according to the size of detectable primary lesions and severity and extent of OA observed; a score of 0 denotes that the elbow is radiographically normal, (1) that signs of mild OA are visible, (2) that a moderate or a primary lesion is present but with no OA, and (3) that there is severe osteoarthritis or a primary lesion with signs of OA. Only the score of the higher elbow grade is publically reported. Pedigree and phenotypic data for the listed breeds were extracted from the Kennel Club electronic databases on 1st April 2019. The EBVs for hip score and elbow grade are re-calculated regularly four times per year using updated pedigree

and phenotypic data and are publically accessible via the Kennel Club website. The calculation of best linear unbiased predictor (BLUP) EBVs is as described by Lewis et al. (22, 25), with genetic parameters estimated using ASREML (32) and the BLUP EBVs calculated using MiXBLUP software (https://www.mixblup.eu/index.html). EBVs were retrieved from files generated in their most recent routine update (April, 2019) and used to examine genetic trends.

Analysis

For each breed included, the number of registered animals born, the number with a hip score; and the median and mean averages, standard deviation, and 75th percentile of those hip scores, each year from 1990 to 2018 inclusive were calculated. Since dogs are required to be over 1 year (365 days) old to participate in the BVA/KC hip (and elbow) screening scheme, the majority of the dogs born in 2018 will have been too young to participate at the time of data extraction (1st April 2019), and so phenotypic data from individuals born in this year was incomplete. Furthermore, given the developmental nature of the disease, younger dogs are known to have lower scores due to less severe pathology (33). Therefore, there is the potential for bias in the scores of cohorts of dogs which are younger, that is born in recent years; e.g., dogs born in 2017 will have been between 15 and 27 months old at time of data extraction, and so scores from older dogs in this cohort are missing, which may bias the parameters. Over 90% of dogs of these breeds are scored before they are 4 years old, so an attempt to minimize potential bias introduction was made by excluding cohorts of 2017, 2016, and 2015 born dogs (which will all have contained dogs under 4 years old at the time of data extraction, and so susceptible to bias). Thus, although the total data extracted comprised dogs born in years up to and including 2018, the score parameters described above of individual 2015–2018 born dogs were excluded from analysis. Therefore, the dataset of hip score parameters on individual registered dogs per year of birth consisted of those dogs born from 1990 to 2014. However, because EBVs are calculated for all animals in the pedigree, including those without phenotypes, analyses were performed on EBV data on dogs born from 1990 to 2018 (the last complete calendar year). EBVs are centered and scaled according to breed-specific parameters from the previous 10 years to give a mean of zero and a standard deviation of ±20, with negative numbers indicating lower genetic risk than 10 year average in the breed.

Finally, per year of birth, the sires and dams of registered animals born were identified, and the proportion of each (sires and dams) with a hip score determined, and the median and mean averages, standard deviation, and 75th percentile of those hip scores calculated. Again, data comprised sires and dams of dogs born from 1990 to 2018.

General analyses of elbow grades included data on individual dogs from the six breeds described born each year from 1998 to 2014 for the same reasons outlined above. The parameters calculated included the proportion of graded dogs, the proportion of total elbow grades (left + right elbow grades) that were zero, and the median, mean and standard deviation of total elbow grade. EBV data on individuals born 1990–2018, and the proportion of sires and dams (of registered dogs born 1990–2018) with elbow grades, and the proportion of total elbow grades equal to zero, were analyzed.

Three year rolling means of parameters over latter years were calculated to provide most recent observed levels for across breed comparison. Linear regression of each parameter of hip score or elbow grade (e.g., mean hip score) on individual year of birth were performed using Matlab (34), and the coefficients (trends) and statistical significance reported along, in some cases, with the R-squared value, which is the proportion of variation in the dependent variable explained by the progressing year of birth.

RESULTS

Hips

Individual Score Parameters Over Year of Birth

There was variation across the six breeds in the proportion of registered animals born per year that had undergone hip screening and so had hip scores, the 3 year rolling mean proportion over 2012–2014 being 7.83% in LR, 10.62% in GR, 8.39% in GSD, 10.05% in ROTT, 18.80% in BMD, and 13.98% in NEWF. Results from linear regression of the percentage of registered animals born that have undergone screening on individual year of birth from 1990 to 2014 revealed varied coefficients; there were statistically significant negative trends in three breeds, LR (−0.0796%), BMD (−0.2546%), and NEWF (−0.3996%) and statistically significant positive trends in the GR (+0.0948%) and GSD (+0.0732%), with no significant trend in ROTT. However, none of the detected regression coefficients were large in magnitude (**Table 1**). Raw data on the proportion of registered animals born per year with hip scores are given in individual breed tables in **Supplementary Table 1**.

The 3 year rolling means of the median hip score of dogs born 2012–2014 were all within a narrow range: 9.00 in LR, 10.33 in GR, 11.00 in GSD, 7.33 in ROTT, 9.00 in BMD, and 10.00 in NEWF (raw data provided in **Supplementary Table 1**). Regression of the median hip score of animals born per year on year of birth from 1990 to 2014 yielded negative (improving) and significant ($P < 0.001$) trends/coefficients in all breeds (**Table 1**), ranging in magnitude from −0.0885 (ROTT) to −0.5165 (NEWF), equating to declines of −2.2 (ROTT) and −12.9 (NEWF) in median hip score over 1990–2014.

The 3 year rolling means of the mean hip score of dogs born per year 2012–2014 were 10.82 for LR, 12.85 for GR, 14.62 for GSD, 9.48 for ROTT, 11.63 for BMD, and 15.19 for NEWF. In all breeds there were more pronounced changes in the mean than for the median; reflecting the skewed distribution of hip scores. Regression of mean hip score from dogs born per year on year of birth from 1990 to 2014 showed a significant, negative (declining) trend/coefficient in all breeds (**Table 1**), ranging from −0.1519 in ROTT ($P < 0.001$) to −0.6353 in NEWF ($P < 0.001$), equating to declines of −3.8 (ROTT) and −15.9 (NEWF) in mean hip score over 1990–2014.

The 3 year rolling means of standard deviation (SD) of hip scores of dogs born 2012–2014 were 9.78 for LR, 9.28 for GR,

TABLE 1 | Regression coefficients (describing trend) of hip score parameters listed on year of birth across breeds, and statistical significance of the trend ($^{ns}P > 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) (sd, standard deviation; pc, percentile).

	LR	GR	GSD	ROTT	BMD	NEWF
Percent scored	−0.0796%**	0.0948%***	0.0732%**	−0.0822%*ns*	−0.2546%***	−0.3996%***
Median score	−0.1162***	−0.1869***	−0.1069***	−0.0885***	−0.1342***	−0.5165***
Mean score	−0.2728***	−0.3208***	−0.2328***	−0.1519***	−0.2799***	−0.6353***
sd score	−0.2361***	−0.2439***	−0.2081***	−0.1517***	−0.2604***	−0.3418***
75th pc score	−0.2900***	−0.4762***	−0.3708***	−0.1408***	−0.3964***	−1.3148***

12.42 for GSD, 8.22 for ROTT, 10.34 for BMD, and 15.13 for NEWF. Regression of standard deviation of hip scores of dogs born per year on year of birth from 1990 to 2014 yielded negative, statistically significant coefficients in all breeds (**Table 1**), ranging from −0.1517 ($P < 0.001$) in ROTT to −0.3418 ($P < 0.001$) in NEWF, implying a reduction in variance of hip scores of dogs born per year from 1990 to 2014, equating to declines of −3.8 (ROTT) and −8.5 (NEWF) in standard deviation of hip score over 1990–2014.

The 3-year rolling means of the 75th percentile hip score of those dogs born 2012–2014 were 11.00 for LR, 13.00 for GR, 14.00 for GSD, 10.17 for ROTT, 12.25 for BMD, and 15 for NEWF Regression of the 75th percentile hip score of dogs born per year on year of birth from 1990 to 2014 showed statistically significant declining trends in all breeds (**Table 1**), ranging from −0.1408 in ROTT ($P < 0.001$) to −1.3148 in NEWF ($P < 0.001$), equating to declines of −3.5 (ROTT) and −32.9 (NEWF) in 75th percentile hip score over 1990–2014.

Regression coefficients for mean, standard deviation and 75th percentile of hip score were greater in magnitude than those for the median hip score (except that for standard deviation in NEWF). These parameters are affected by the skew in the distribution and so the larger declining trends compared to the median imply fewer higher scores and so a contraction of the skewed "tail" of the distribution of hip scores. Raw data on the median, mean, standard deviation and 75th percentile hip scores of registered animals born per year are given in individual breed tables in **Supplementary Table 1**.

EBV/Genetic Trend Over Year of Birth

The mean EBV for dogs of each breed born per year is shown in **Table 2**.

All breeds show a declining trend in mean EBV for hip score of dogs born per year from 1990 to 2018 (**Table 2**). Regression of mean EBV on year of birth showed declining trends in all breeds: −1.2900 in LR, −1.3655 in GR, −0.9514 in GSD, −0.8894 in ROTT, −0.7732 in BMD, and −0.9038 in NEWF. All regression coefficients were statistically significantly different to zero ($P < 0.001$).

TABLE 2 | Mean EBV (10 year mean = 0, standard deviation = ±20) of dogs born per year across breeds.

YoB	LR	GR	GSD	ROTT	BMD	NEWF
1990	30.73	33.53	20.95	23.42	21.47	26.47
1991	29.48	31.30	20.13	21.36	18.25	23.89
1992	28.06	31.37	20.30	17.80	17.99	19.31
1993	26.97	28.97	19.04	18.69	15.10	19.75
1994	25.58	26.67	19.04	17.31	13.18	19.57
1995	24.03	25.37	18.37	14.75	13.90	14.36
1996	22.07	23.50	17.43	13.70	10.94	13.94
1997	20.38	22.28	17.10	12.90	10.47	12.16
1998	18.61	19.84	15.72	13.67	12.42	12.81
1999	17.00	18.65	14.02	12.20	8.79	9.51
2000	16.91	17.93	13.10	11.79	10.89	7.72
2001	15.48	15.78	13.04	10.65	8.60	8.43
2002	14.14	14.20	12.15	10.10	8.19	5.56
2003	12.69	12.07	11.50	10.27	10.12	7.12
2004	11.50	12.14	9.61	8.55	6.94	4.80
2005	10.79	11.01	8.69	9.12	3.12	2.64
2006	8.97	9.00	7.43	7.73	6.69	4.27
2007	7.96	7.31	5.54	6.31	3.20	0.33
2008	6.83	5.43	3.96	6.41	4.13	−0.62
2009	5.08	4.30	1.81	3.63	2.00	−1.37
2010	3.62	3.07	2.50	2.99	2.86	−1.60
2011	2.95	1.81	1.78	0.59	1.71	−0.84
2012	1.24	1.26	0.89	1.59	2.10	−0.21
2013	0.84	−0.56	0.29	0.73	−0.53	1.74
2014	−0.84	−1.36	−0.95	−1.64	−1.09	0.74
2015	−1.22	−1.71	−0.57	−2.57	−1.16	1.95
2016	−2.91	−1.69	−1.32	−3.92	−2.06	1.91
2017	−4.25	−2.17	−2.63	−0.95	−3.41	0.30
2018	−5.87	−2.79	−3.79	−3.36	−1.36	−2.95

Note that EBVs are calculated for all dogs in a pedigree, regardless of whether they have phenotypic records or not (although the accuracies of EBVS—not shown—will generally be greater for dogs with phenotypic records and/or with multiple close relative with phenotypic records).

Score Parameters of Sires and Dams of Dogs Born Over Year of Birth

There was variation across breeds in the proportion of sires and dams (of dogs born per year) that have undergone hip screening, with the 3 year rolling means of the proportion of sires and dams, respectively with scores over 2016–2018 being: 56.24% and 56.93% in LR, 69.58% and 73.20% in GR, 43.93% and 50.87% in GSD, 47.06% and 51.85% in ROTT, 49.28% and 53.59% in BMD, and 51.18% and 63.19% in NEWF. Regression coefficients over year of birth of the proportion of sires and dams scored were positive (implying an increase) and statistically

TABLE 3 | Regression coefficients (regr.coef) (describing trend), R-squared value (R-sq) (describing proportion of variance in the dependent variable that is accounted for by the independent variable) and statistical significance of the trend ($^{ns}P > 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) of the percentage of screened (i.e., with hip scores) sires and dams of dogs born per year regressed on year of birth.

	Sires			Dams		
	Regr. coef.	**R-sq**	**Significance**	**Regr. coef.**	**R-sq**	**Significance**
LR	1.2112	0.6681	***	1.1757	0.5622	***
GR	1.6714	0.5758	***	1.7251	0.5640	***
GSD	1.0319	0.6223	***	1.3277	0.7053	***
ROTT	0.9913	0.4951	***	1.1779	0.5504	***
BMD	0.1472	0.0038	ns	0.2715	0.0110	ns
NEWF	0.7430	0.1616	*	1.1514	0.2433	**

Negative regression coefficients indicate at declining trend, and positive an increasing trend, and magnitude of ±1.00 implies a trend of increase/decrease of 1% in the proportion of that category per progressive year of birth.

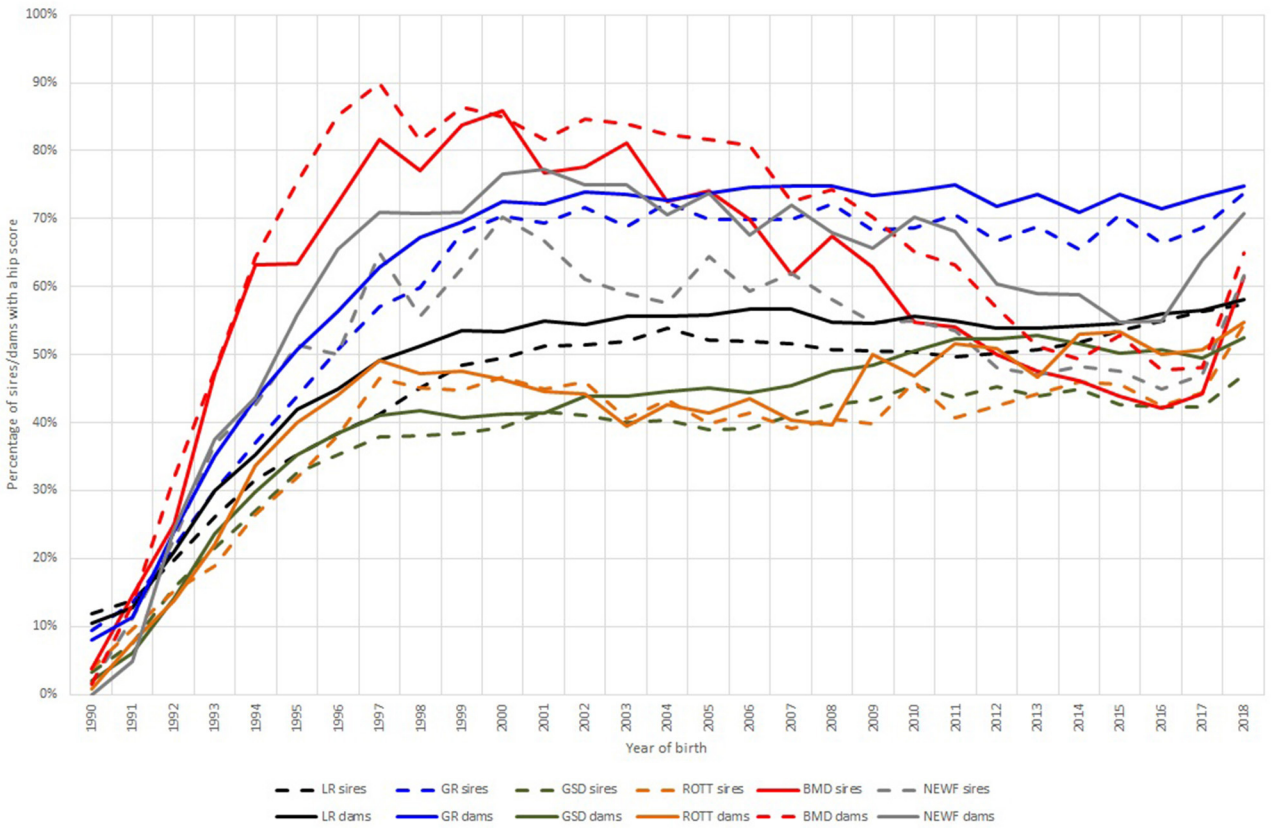

FIGURE 1 | The percentage of sires (dashed lines) and dams (solid lines) (of dogs born per year) that have undergone screening and so have a hip score, in Labrador Retrievers (LR—black), Golden Retrievers (GR—blue) and German Shepherd Dogs (GSD—green), Rottweiler (ROTT—orange), Bernese Mountain Dog (BMD—red), and Newfoundland (NEWF—gray). The figure illustrates that the percentage "plateaus" in the late 1990s/early 2000s.

significant in all breeds except BMD (**Table 3**). However, non-linearity due to a "plateauing" of the proportion of sires and dams scored, which occurred in all breeds in the late 1990s/early 2000s (**Figure 1**) would have reduced the magnitude of the overall regression coefficient/trend detected, and the R-squared value, compared to a continued rate of change to that observed in earlier years. Raw data on the hip scores of sires and dams of registered dogs born per year are given in individual breed tables in **Supplementary Table 2**.

There was variation across breeds in the 3 year rolling means of hip score parameters of sires and dams of dogs born over 2016–2018 (**Table 4**).

The regression coefficients/trends determined in median, mean, standard deviation, and 75th percentile of sire and dam

TABLE 4 | Three-year rolling average of hip score parameters (median, mean, standard deviation, 75th percentile of sires and dams of dogs born in 2016–2018.

		LR	GR	GSD	ROTT	BMD	NEWF
Hip score parameters of sires of 2016–2018 born dogs	Median	8.00	10.00	10.00	6.33	8.67	9.83
	Mean	8.37	10.53	10.97	7.14	9.93	10.91
	sd	5.05	4.35	5.75	3.36	6.61	5.93
	75th percentile	10.00	12.00	12.00	9.00	11.33	12.33
Hip score parameters of dams of 2016–2018 born dogs	Median	9.00	11.00	12.00	8.00	10.00	9.67
	Mean	9.32	12.18	13.85	9.58	11.18	13.09
	sd	5.52	6.09	9.24	6.73	6.07	11.03
	75th percentile	11.00	14.00	15.00	10.33	13.00	13.67

TABLE 5 | Regression coefficients (describing trend) and statistical significance of the trend ($^{ns}P > 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) of the median, mean, standard deviation and 75th percentile of hip score of sires and dams of dogs born per year regressed on year of birth.

		LR		GR		GSD		ROTT		BMD		NEWF	
Median score	Sires	−0.0079	ns	−0.0271	ns	0.0099	ns	−0.0404	**	−0.0182	ns	−0.1089	**
	Dams	−0.0133	ns	−0.0099	ns	0.0502	*	0.0138	ns	0.0219	ns	−0.2815	***
Mean score	Sires	−0.1014	***	−0.0930	***	−0.0719	**	−0.0560	**	−0.0501	ns	−0.3393	***
	Dams	−0.1037	***	−0.0660	ns	−0.0468	***	0.0174	ns	−0.0539	ns	−0.3354	***
sd score	Sires	−0.2242	***	−0.1913	***	−0.2521	***	−0.1510	***	−0.0950	ns	−0.4262	**
	Dams	−0.2110	***	−0.1595	***	−0.1880	***	0.0493	*	−0.1141	ns	−0.1853	*
75th pc score	Sires	−0.0773	*	−0.1025	*	−0.0853	*	−0.0520	*	−0.0557	ns	−0.6063	***
	Dams	−0.0537	ns	−0.0729	ns	−0.0390	ns	−0.0100	ns	−0.0766	ns	−0.6318	***

Negative regression coefficients indicate at declining trend, and positive an increasing trend.

hip score over year of birth are shown in **Table 5**. Regression of median hip score on year of birth determined trends that were negative (indicating decline) and statistically significant in just a few instances; ROTT sires (−0.0404, $P < 0.01$), NEWF sires and dams (−0.1089, $P < 0.01$; −0.2815, $P < 0.001$), and were positive and statistically significant in GSD dams (0.0502, $P < 0.05$, **Table 5**).

A greater number of statistically significant declining trends were determined from regression of sire and dam mean hip score (**Table 5**), ranging from −0.0468 (GSD dams) to −0.3393 (NEWF sires).

For standard deviation of sire and dam hip score, all trends were negative and statistically significant, ranging from −0.1510 (ROTT sires) to −0.4262 (NEWF sires), with the exception of ROTT dams, which was a statistically significant positive (increasing) trend, and BMD sires and dams which were not statistically significant. For the 75th percentile of sire hip scores, all breeds had a statistically significant declining trend (ranging from −0.0520 for ROTT to −0.6063 for NEWF), except for BMD. For dams none of the breeds had a statistically significant trend in 75th percentile hip score, except for NEWF (−0.6318, **Table 5**). Raw data on the hip scores of sires and dams of registered dogs born per year are given in individual breed tables in **Supplementary Table 2**.

Summary of Changes in Hip Scores

A summary table showing the detection of statistically significant ($P < 0.05$), favorable (improving) trends in various hip score parameters of individuals and sires and dams over progressing year of birth is shown in **Table 6**. In most breeds there is some evidence of some improvement.

The generally larger change in parameters affected by the skewed nature of the distribution of hip score implies that improvement has taken the form of a reduction in this skew. This can be observed as a contraction in the long "tail" of high scores on the right hand side of the distribution when comparing the distribution of hip scores from 1990 to 1992 vs. 2012 to 2014 born NEWF and LR (**Figure 2**).

Elbows

Individual Grade Parameters Over Year of Birth

There was variation across the six breeds in the proportion of registered animals born per year that had undergone screening and so had elbow grades, with the 3 year rolling mean proportion over 2012–2014 being: 5.57% in LR, 6.99% in GR, 6.35% in GSD, 7.54% in ROTT, 18.32% in BMD, and 12.26% in NEWF. Linear regression of the percentage of registered animals born that have undergone screening on individual year of birth from 1998 to 2014 determined a significant increasing trend in all breeds (**Table 7**). However, none of the detected regression coefficients were particularly large in magnitude.

The rolling means of the percentage of total (left + right) elbow grades that were zero in dogs born 2012–2014 were 87.29% in LR, 77.27% in GR, 81.29% in GSD, 49.07% in ROTT, 64.27% in BMD, and 69.83% in NEWF. Regression of the proportion of zero grades in dogs born per year on year of birth was only

TABLE 6 | Summary of regression coefficients of the parameters of hip score on year of birth across breeds, as described in the results.

		LR	GR	GSD	ROTT	BMD	NEWF
Individuals	% with hip score						
	Median hip score						
	Mean hip score						
	sd hip score						
	75th percentile hip score						
	EBV hip score						
Sires	% with hip score						
	Median hip score						
	Mean hip score						
	sd hip score						
	75th percentile hip score						
Dams	% with hip score						
	Median hip score						
	Mean hip score						
	sd hip score						
	75th percentile hip score						

Where the regression coefficient was both favorable, implying improvement (for example increasing percentage with scores, or decreasing mean or standard deviation of scores) and statistically significant, it is indicated in green. When either unfavorable, or not statistically significant (or both), this is indicated in red.

statistically significant for LR (**Table 7**), equating to an increase of approximately +0.4% per year.

Median total elbow grades were predominantly zero reflecting the preponderance of the zero grade, and so were not considered here.

The 3 year rolling mean of mean total elbow grade of dogs born over 2012–2014 were 0.2732 in LR, 0.4780 in GR, 0.4313 in GSD, 1.2142 in ROTT, 1.0130 in BMD, and 0.7620 in NEWF. Regression of mean total elbow grade of animals born per year on year of birth from 1998 to 2014 determined declining trends significantly different to zero in LR (−0.0106), GSD (−0.0119), ROTT (−0.0242), and BMD (−0.0214, **Table 7**).

For standard deviation of total elbow grade per year of birth, the 3-year rolling means over 2012–2014 per breed were: 0.8581 for LR, 1.0342 for GR, 1.0430 for GSD, 1.455 for ROTT, 1.6494 for BMD, and 1.4022 for NEWF. Regression of standard deviation of total elbow grade of animals born per year on year of birth determined that the trend was significant in only 3 breeds (−0.0136 in LR; −0.0083 GR; −0.0141 in BMD). Raw data giving the parameters of total elbow grades are given in individual breed tables in **Supplementary Table 3**.

EBV/Genetic Trend Over Year of Birth

Mean EBVs for elbow grade of dogs born per year are shown in **Table 8**. Regression of mean EBV of dogs born per year on year of birth were significant for all breeds except NEWF; −0.6381 ($P < 0.001$, LR), −0.0976 ($P < 0.05$, GR), −0.6828 ($P < 0.001$, GSD), −0.9283 ($P < 0.001$, ROTT), and −1.157 ($P < 0.001$, BMD).

Grade Parameters of Sires and Dams of Dogs Born Over Year of Birth

The proportions of sires and dams of animals born per year which have an elbow grade were notably higher in BMD than other breeds in 1998–2000, with 3 year rolling mean of 22.25% and 22.45%, respectively [vs. 0.15% (GSD, dams) to 3.63% (GR, sires)]. However, by 2016–8 the disparity in the proportion of graded sires and dams across breeds had disappeared. For sires the percent graded over 2016–2018 born animals were 41.15% for LR, 48.83% for GR, 34.17% for GSD, 34.14% for ROTT, 47.80% for BMD, and 46.64% for NEWF. For dams the equivalent figures were 39.63% for LR, 45.73% for GR, 39.73% for GSD, 35.68% for ROTT, 53.12% for BMD, and 56.06% for NEWF. Regression of proportions of sires and dams with elbow grades on year of birth from 1998 to 2014 showed significant increasing trends in all breeds, except in BMD (**Table 9**).

The 3 year rolling means of proportion of total elbow grades that were grade zero for sires and dams of dogs born over 2016–2018 were 92.76% and 91.66% in LR, 88.17% and 81.90% in GR, 86.32% and 86.94% in GSD, 44.14% and 54.64% in ROTT, 82.69% and 61.39% in BMD, 64.21% and 76.86% in NEWF. Results of regression over year of birth of the proportion of zero grades of sires and dams are given in **Table 9**. For sires, significant positive trends were observed for LR (0.72% increase per year), GR (0.20% increase per year), ROTT (2.04% increase per year), BMD (0.80% increase per year) and NEWF (2.13% increase per year). For dams, significant positive trends were observed for LR (0.48% increase per year) and GR (0.77% increase per year). Raw data on the total elbow grade parameters of sires and dams of registered dogs born per year are given in individual breed tables in **Supplementary Table 4**.

Summary of Changes in Elbow Grades

A summary table showing the detection of statistically significant ($P < 0.05$), favorable (improving) trends in various elbow grade parameters of individuals and sires and dams over progressive year of birth is shown in **Table 10**.

DISCUSSION

This analysis of data from canine hip and elbow dysplasia screening schemes in the UK has demonstrated improvements in participation, phenotypic parameters and/or genetic trends for all breeds considered. Generally, greater progress was observed with respect to hip scores than elbow grades. The largest improvements in hip score data were observed in NEWF, which initially had the highest (poorest) scores. For some of the very popular breeds, for which hip dysplasia is a recognized problem (LR, GR, GSD), steady improvement was observed. In general, the changes observed in elbow grade parameters were less consistent and smaller although there were general increases detected in participation across breeds and an improving genetic trend was detected in five of the six breeds included. However, the genetic trend as determined by elbow grade EBVs was comparable with that for hip score in ROTT and exceeded it in BMD, perhaps revealing selection priorities of breeders.

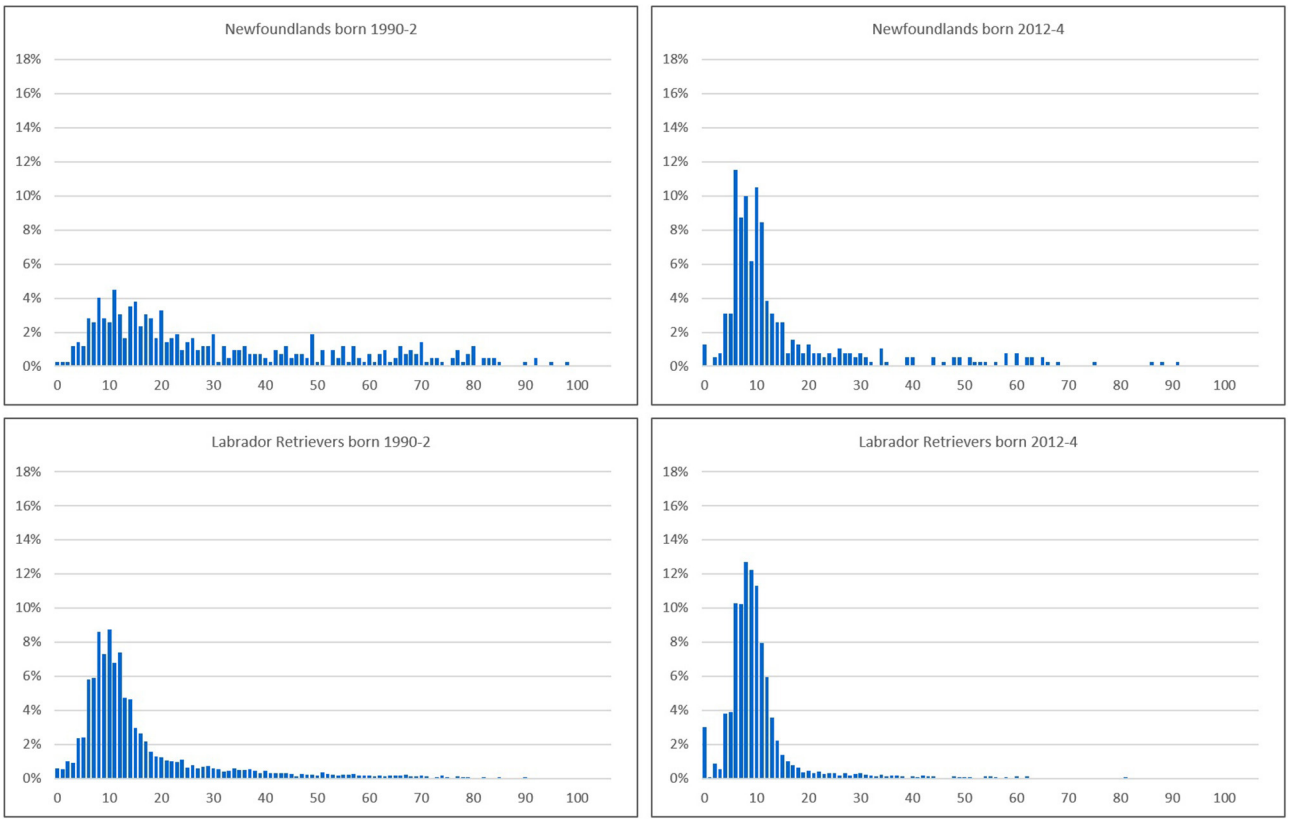

FIGURE 2 | Distributions of hip scores from dogs born in 1990–2 (**Left**) and 2012–4 (**Right**) in two breeds: the NEWF (**Top**) exhibiting a major change, and the LR (**Bottom**) showing a moderate reduction in the skew/contraction in the "tail" of the distribution.

TABLE 7 | Regression coefficients (describing trend) of elbow grade parameters listed on year of birth across breeds, and statistical significance of the trend ($^{ns}P > 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

	LR		GR		GSD		ROTT		BMD		NEWF	
Proportion scored	0.3565%	***	0.4420%	***	0.4750%	***	0.5314%	***	0.1987%	*	0.8738%	***
% zero	0.3778%	***	0.0221%	ns	0.1723%	ns	0.7382%	ns	0.4124%	ns	−0.1472%	ns
Mean score	−0.0106	***	−0.0032	ns	−0.0119	*	−0.0242	*	−0.0214	*	−0.0094	ns
sd score	−0.0136	**	−0.0083	**	−0.0238	ns	−0.0034	ns	−0.0141	*	−0.0166	ns

The findings from this analysis of generally improving phenotypic and genetic trends are consistent with those reported in these and other breeds in a range of other countries sometimes with different evaluation schemes (12, 23, 24, 35). This indicates that selection has initiated a positive shift in assessments of hip and elbow health over time, whatever the specific details of the hip screening phenotype (phenotypes of elbow screening being more consistent). However, a recent study has reported a persisting risk of hip OA, as judged by a "distraction index" evaluation, in dogs scored as "excellent" under an "extended view" dysplasia screening scheme (36). This implies that there may be variation in hip laxity (leading to OA) which is not captured by some screening schemes, indicating that betterment of scoring parameters may be necessary to enable further improvement in reducing the ultimate risk of OA. However, selection based on EBVs has been suggested as a method with higher accuracy and so potential to induce improvements more quickly than selection upon phenotype alone, as demonstrated in previous studies (23, 25), and have profound impacts regardless of the parameters specified within a specific scheme (37).

There are several criteria which must be met to describe a screening scheme for heritable disorders as "effective," and so several factors which may be examined to gauge the success or failure of such screening schemes. The first step that must be accomplished is a high general rate of participation, particularly among breeding individuals. This entails both a degree of acknowledgment by breeders that the condition compromises welfare and that it is present in the breed population at a heightened prevalence. Breeders must then accept the costs associated with screening as part of the regular costs of breeding.

The time taken to achieve these steps may vary across different breed populations and be dependent on a number of factors, such as the severity of welfare impairment and the cost of screening (which may vary greatly, e.g., auscultation vs. an MRI scan). Participation in hip scoring, as determined in this analysis, is broadly rising for both dams and sires across most breeds in line with indicators of improvement in hip health, albeit with evidence of plateauing in recent years. Regarding the elbow scheme, five of the six breeds showed significantly rising participation of both sires and dams over time. The exception was the BMD, although it should be noted that this breed began with notably higher participation in the first instance.

The second step in determining efficacy is that, subsequent to participation, the results of screening are used in selection decisions. At first consideration, it might appear absurd that a breeder would undertake the costs of screening only then to ignore the result. However, if there is peer-pressure among contemporaries and wider society to participate in screening, then an individual may decide that being seen to participate is desirable, even if they remain skeptical of the prevalence of the condition, the severity of welfare impact or the relevance to their breeding animals. If understanding of the screening results by the public is poor and the results of screening are not publically available, then this motivation may be heightened as there is less chance of being exposed as not basing breeding decisions on the results of screening. Unfortunately, there is no feasible way of knowing to what extent phenotypic data from screening influence the breeding decisions of breeders (individually or collectively), and so parameters of the phenotypes over time must be analyzed to determine any changes and draw inferences. There were general improving trends in hip score across breeds, with evidence of changes of greater magnitude in parameters that reflect the skewed distribution of hip score, i.e., with a longer "tail" on the right hand side of the distribution (see **Figure 2**). For example, the regression coefficients of 75th percentile of hip score over time were between 1.6 and 3.5 times larger than those of median hip score. Greater rates of improvement in the mean, standard deviation and 75th percentile compared to the median hip score indicate fewer individuals with the high scores indicative of severe OA occurred over time. The changes

TABLE 8 | Mean elbow grade EBV (10 year mean = 0, standard deviation = ±20) of dogs born per year across breeds.

YoB	LR	GR	GSD	ROTT	BMD	NEWF
1998	8.64	2.40	9.00	12.28	19.65	3.57
1999	8.22	2.48	8.86	11.25	17.96	2.34
2000	7.98	1.06	8.30	10.80	16.10	2.90
2001	7.44	0.75	9.16	10.49	12.07	0.17
2002	7.43	0.65	9.06	10.51	11.71	−0.37
2003	6.54	−0.11	7.63	9.83	9.25	−0.67
2004	6.54	−0.98	6.56	9.52	11.68	1.70
2005	5.87	0.41	6.15	8.79	4.93	1.43
2006	5.08	−0.30	5.07	8.23	8.30	−1.26
2007	4.73	−0.26	4.77	8.11	8.85	−0.56
2008	4.27	0.14	3.80	7.50	5.48	−0.71
2009	3.83	−0.05	3.28	5.00	5.15	−1.68
2010	2.90	0.96	2.65	5.67	4.48	0.92
2011	2.41	−0.05	1.48	2.04	3.41	0.30
2012	0.83	1.38	0.59	3.21	1.17	−1.05
2013	0.49	0.45	0.08	0.48	1.11	−0.13
2014	−0.66	1.95	−0.95	−0.93	−0.93	−0.83
2015	−1.08	−0.13	−1.90	−3.83	−4.74	−0.27
2016	−2.40	−1.35	−2.29	−3.25	−4.55	1.59
2017	−2.92	−0.88	−2.40	−4.93	−2.73	1.33
2018	−4.48	−1.68	−3.03	−6.46	−5.07	0.89

Note that EBVs are calculated for all dogs in a pedigree, regardless of whether they have phenotypic records or not (although the accuracies of EBVS—not shown—will generally be greater for dogs with phenotypic records and/or with multiple close relative with phenotypic records).

TABLE 10 | Summary of regression coefficients of the parameters of elbow grade on year of birth across breeds as described in the results.

		LR	GR	GSD	ROTT	BMD	NEWF
Individual	% with elbow grade						
	% zero grade						
	Mean elbow						
	sd elbow grade						
	EBV elbow grade						
Sire	% with elbow grade						
	% zero grade						
Dam	% with elbow grade						
	% zero grade						

Where the regression coefficient was both favorable implying improvement (for example increasing percentage with grades, or decreasing mean or variance of grades) and statistically significant it is indicated in green. When either unfavorable, or not statistically significant (or both), this is indicated in red.

TABLE 9 | Regression coefficients (describing trend) and statistical significance of the trend ($^{ns}P > 0.05$; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) of the percentage of sires and dams of dogs born per year with elbow grades, and the percentage of sire and dam elbow grades that were zero, regressed on year of birth.

		LR	GR	GSD	ROTT	BMD	NEWF
% with grade	Sires	2.19%***	2.57%***	2.00%***	1.92%***	0.91% ns	2.70%***
	Dams	2.13%***	2.37%***	2.25%***	2.04%***	0.98% ns	3.06%***
% grade zero	Sires	0.72%***	0.20%*	0.39% ns	2.04%***	0.80%*	2.13%*
	Dams	0.48%***	0.77%**	0.70% ns	−0.83% ns	0.59% ns	−0.18% ns

Negative regression coefficients indicate at declining trend, and positive an increasing trend, and magnitude of ±1.00 implies a trend of increase/decrease of 1% in the proportion of that category per progressive year of birth.

appeared greatest in breeds which had the worst scores in the early 1990s (NEWF).

With regard to elbow grade the evidence of improving phenotypes was less consistent. The only breed showing steady improvement in all parameters of elbow grade (% zero grade, mean and standard deviation) was the LR, although there were significant declining trends in mean and standard deviation of elbow grade in the BMD, in mean grade in GSD and ROTT, and in standard deviation of grade in GR. The declining trends in mean elbow grade were notably larger in magnitude in ROTT and BMD than other breeds. ROTT and BMD also had markedly higher (worse) mean grades in early years, again supporting the suggestion that incidence and severity are motivators for improvement. The reported estimates of heritability for elbow score have consistently been lower than those for hip score (12, 18, 19, 25, 38), which would result in smaller genetic improvements in elbow grade than compared to hip score at the same selection intensity. Potential reasons for the lower heritability of elbow grade will include the categorical nature of the grade compared to the more continuous hip score, with each category potentially encompassing much variation in degree of pathology (particularly a grade of zero), and the plurality of individual subsets of disease included which may reduce specificity.

Improvements in the prevalence and severity of complex disease in a population, however, may come via a number of different routes, reflecting the multifactorial etiology of which genetics is just one (albeit often the single largest) contributing influence. For example, it could be that the general improvements in hip scores observed are achieved via a greater understanding of the effects of feed intake and levels of exercise in young dogs (10, 11), and subsequent appropriate changes to management. To infer selection is contributing to progress, therefore, it is necessary to examine any changes in the phenotypes of breeding animals over time, and to determine genetic changes it is necessary to examine the trend of EBVs. There were general improvements in participation in, and most parameters of, hip scoring for sires and dams across most breeds. Where the evidence of improving hip score parameters in sires and dams was weaker, despite improving trends in individual parameters and EBVs (e.g., ROTT dams, BMD), the small numbers of sires and dams with scores in early years may have had a disruptive influence in detecting trends (see **Supplementary Table 2**). It is possible that where participation was very low in the early years included in this study, those participating breeders may have been "early-adopters" and promoters of hip screening in these breeds, and so may also have been including some indicator of hip health in prior selection strategies. This could have introduced a bias to the data from early years, and a truer representation of the parameters may be found a few years later, when participation in screening was more the norm among sires and dams, and so the sample is more representative. While there were increases, or maintained high levels, in sire and dam participation in elbow screening across the six breeds, improvement in the proportion of which were grade zero was less consistent, possibly due to the categorical nature of grades and the preponderance of zero grades. Nevertheless, there was a significantly improving trend in proportion of at least either sire or dams with total elbow grade of zero in all breeds, except the GSD.

Trends in EBVs for hip score were favorable in all breeds, and for EBVs of elbow grade they were favorable for all breeds, except NEWF. There is no obvious reason that stands out as to why, despite an improving rate of participation of sire and dams in elbow screening (in-line with, or latterly exceeding, most of the other breeds), there were no detectable improving trends in elbow grade in this breed. The absolute numbers of sires and dams with an elbow grade was in single figures until 2006 (dams) and 2007 (sires), perhaps revealing a slow initial uptake in the breed. Under the hypothesis that initial participants in the scheme may exert a downward bias to phenotypic severity, and that gradual improved participation with the resultant use of phenotypes guiding selection, it might be expected that the elbow grades will begin to improve over an extended period of time. With regard to the remaining breeds, the generally improving genetic trend, along with a general improvement in screening participation and parameters of sires and dams implies that selection is being applied, giving rise to a consequential improvement in population-wide genetic risk.

The magnitude of the genetic response can be directly compared across breeds and phenotypes, since EBVs are centered and scaled by the mean and standard deviation in the breed over the previous decade, to give a 10 year mean EBV of zero and [genetic] standard deviation ± 20. It is therefore possible to determine that, for example, the genetic progress in LR with respect to hip scores was approximately twice that for elbow grades (regression coefficients of -1.29 vs. -0.64). In these six breeds with EBVs for both hip score and elbow grade, the genetic trend was higher for hip score than elbow grade for LR, GR, GSD, and NEWF, but higher for elbow grade for ROTT and BMD, perhaps reflecting breeder objectives. The genetic trends imply that, in most breeds, selection is being applied based on the results of screening, and a genetic response elicited.

In conclusion, this study has demonstrated evidence of improving genetic trends with respect to hip score and elbow grade in six UK registered breeds in line with phenotypic improvements and participation in screening schemes. In general, improvement tends to be greater for hip score than elbow grade. This is possibly due to longstanding concerns over hip dysplasia and a more established screening scheme and culture of participation (at least in some breeds). Higher heritability estimates, due perhaps to genetic etiology but also maybe to the quantification of pathology to some degree, will also have played a role in this disparity in rates of improvement. There is variation across breeds in both the apparent prevalence of disease and the rates of improvement. Breeds with poorer hip scores or elbow grades at the outset of the periods included in this study tended to show the greatest rates of improvement.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

Data used in this study were measures from screening schemes to which owners voluntarily submitted their dogs. There was no research on animals, humans, or inclusion of identifiable human data.

AUTHOR CONTRIBUTIONS

FM and TL organized the data. HJ and TL performed the statistical analysis. All authors contributed conception and design of the study, manuscript revision, wrote sections of the manuscript, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2019.00490/full#supplementary-material

Supplementary Table 1 | Hip score statistics of individuals born per year by breed.

Supplementary Table 2 | Hip score statistics of sires and dams of dogs born per year by breed.

Supplementary Table 3 | Elbow grade statistics of individuals born per year by breed.

Supplementary Table 4 | Elbow grade statistics of sires and dams of dogs born per year by breed.

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SYSTEMATIC REVIEW
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Risk Factors for Canine Osteoarthritis and Its Predisposing Arthropathies: A Systematic Review

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Osteoarthritis is a common clinical and pathological end-point from a range of joint disorders, that ultimately lead to structural and functional decline of the joint with associated lameness and pain. Increasing understanding of the risk factors associated with osteoarthritis will assist in addressing the significant threat it poses to the welfare of the dog population and implementing preventive measures. Presented here, is the first comprehensive systematic review and evaluation of the literature reporting risk factors for canine osteoarthritis. This paper aimed to systematically collate, review and critically evaluate the published literature on risk factors for canine osteoarthritis and its predisposing conditions such as developmental joint dysplasias, cruciate ligament degeneration, and patellar luxation. Peer-reviewed publications were systematically searched for both osteoarthritis and predisposing arthropathies on Web of Science and PubMed following PRISMA (2009) guidelines, using pre-specified combinations of keywords. Sixty-two papers met the inclusion criteria and were evaluated and graded on reporting quality. Identified risk factors included both modifiable factors (neuter status and body weight) for which intervention can potentially affect the risk of occurrence of osteoarthritis, and unmodifiable factors (sex, breed, and age) which can be used to identify individuals most "at risk." Osteoarthritis in dogs frequently develops from predisposing arthropathies, and therefore risk factors for these are also important to consider. Papers evaluated in this study were rated as medium to high-quality; gap analysis of the literature suggests there would be significant benefit from additional research into the interactions between and relative weighting of risk factors. There are a number of examples where research outcomes are conflicting such as age and sex; and further investigation into these factors would be beneficial to attain greater understanding of the nature of these risks. Comprehensively collating the published risk factors for osteoarthritis and its predisposing conditions offers opportunities to identify possible means for control and reduction within the population through preventative methods and control strategies. These factors are highlighted here, as well as current literature gaps where further research is warranted, to aid future research direction.

Keywords: canine, dog, degenerative joint disease, osteoarthritis, risk factor, systematic review

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INTRODUCTION

Osteoarthritis, a common pain-causing condition of synovial joints, affects millions of human and non-human animals worldwide (1). Osteoarthritis—otherwise referred to as osteoarthrosis or degenerative joint disease—is a disease of the entire joint organ, including all its associated tissues, but is most frequently associated with the loss and dysfunction of articular cartilage (2). The etiology of osteoarthritis is complex and the specific pathways that lead to its development remain uncertain (3). In humans, reported risk factors for development of osteoarthritis are manifold with both systemic and local causes, linked to factors including: genetics, age, sex, obesity, previous joint trauma, and underlying diseases such as cruciate ligament rupture and osteochondritis dissecans (1). Although osteoarthritis has been reported in a wide range of non-human species, the prevalence of the condition in many of these species remains largely unexplored and as such underreported (4).

With an estimated 9 million pet dogs owned in the UK (5), and 63.4 million households in the US owning a dog (6), the disease burden of osteoarthritis to dogs worldwide is considerable and poses a significant threat to canine welfare. Osteoarthritis prevalence in North America is reported at 20% of all dogs over 1 year of age based on data collected from 200 veterinarians (7). Recent prevalence estimates (likely heavily underestimated due to the nature of reporting methodology) for osteoarthritis in the UK dog population vary widely, from 2.5 and 6.6% of dogs of any age and breed attending primary-care practices [estimates respectively from (8, 9), and up to 20% of dogs over 1 year of age (10)]. In addition to the welfare impact for dogs, canine osteoarthritis is also a major issue worldwide for veterinarians, owners and breeders. Canine osteoarthritis can particularly impact an owner's welfare, with treatment plans having considerable financial costs. For example $1.32bn was spent on cruciate ligament ailments alone in dogs in the US in 2003 (11). There is also the emotional cost to the owner dealing with an animal that is chronically or terminally unwell and/or in chronic pain, which can cause psychological distress and upset known as caregiver burden (12).

Primary osteoarthritis is described as largely idiopathic, but can be associated with several risk factors including aging and obesity (13). Secondary osteoarthritis, where underlying disease processes or injuries play a role in the development of osteoarthritis, is believed to be the most common form in dogs (14). The pathogenesis of secondary osteoarthritis is considered to have a genetic component exacerbated through aspects of lifestyle that impact body condition, such as diet and exercise (15). Disease processes and pre-existing arthropathies often influence the pathogenesis, for example cranial cruciate ligament disease is a common cause of pelvic limb lameness and can result in osteoarthritis development in breeds of all sizes (16). Joint dysplasia, commonly occurring in the hip or elbow, describes failure of normal joint formation during development and can lead to well-recognized and described joint conditions which cause pain and lameness in their own right, and can progress to osteoarthritis (17). Consequently, it is important to understand the risk factors for these complex diseases when considering the epidemiology of canine osteoarthritis.

A critical evaluation of the existing published evidence on risk factors for osteoarthritis and its predisposing conditions is required in order to assess what is known and where the key gaps in knowledge remain. Here, a comprehensive systematic review and evaluation of literature reporting risk factors for canine osteoarthritis is presented. Within this review, the published risk factors associated with the development of both osteoarthritis and predisposing conditions are highlighted, the reporting quality of current evidence is evaluated and recommendations for future research based on existing findings and gaps in knowledge are discussed.

MATERIALS AND METHODS

Literature Search

Stage 1—Identification

The peer-reviewed literature was systematically searched for papers which may have included risk factors associated with canine osteoarthritis and its predisposing conditions, using the approach outlined by the PRISMA (2009) guidelines [(18); **Figure 1**]. The online databases Web of Science (WoS) and PubMed were used to generate broad searches using key topic words within logical sequences incorporating Boolean operators ("AND" and "OR") to ensure papers included (within any part of the paper), the keywords of interest (**Table 1**). All identified papers from each search were stored in a Microsoft Excel database. Data stored included author names, year of publication, paper title, journal title, issue, volume, and page numbers. Literature searches were conducted during March 2019.

Stage 2—Screening

Papers identified during Stage 1 were carried forward to Stage 2. They were initially sorted by title by one researcher (KA); the title had to include reference to dog/canine and osteoarthritis (or a synonym) or an associated disorder (listed in **Table 1**). Included papers had to evaluate at least one risk factor as suggested by the inclusion of words in the title such as but not limited to "risk factor," "prevalence," "predictors," or "susceptibility." Screening stage lists (300 randomly selected papers per reviewer) were independently evaluated by two additional reviewers (1 HZ, 2. LC). Inter-observer reliability (the degree of consistency in selecting the papers between all three researchers) was calculated using percentage of agreement. In the case where there was disagreement, for example where human error occurred, the list was re-reviewed (by KA) and papers were included or discarded upon second review of the title.

Stage 3—Eligibility

Papers retained from Stage 2 moved to Stage 3, which involved firstly checking the abstract for relevance to the inclusion criteria. Papers that were retained through the abstract checks were then read in full and either retained or excluded based on their match to the inclusion criteria described below.

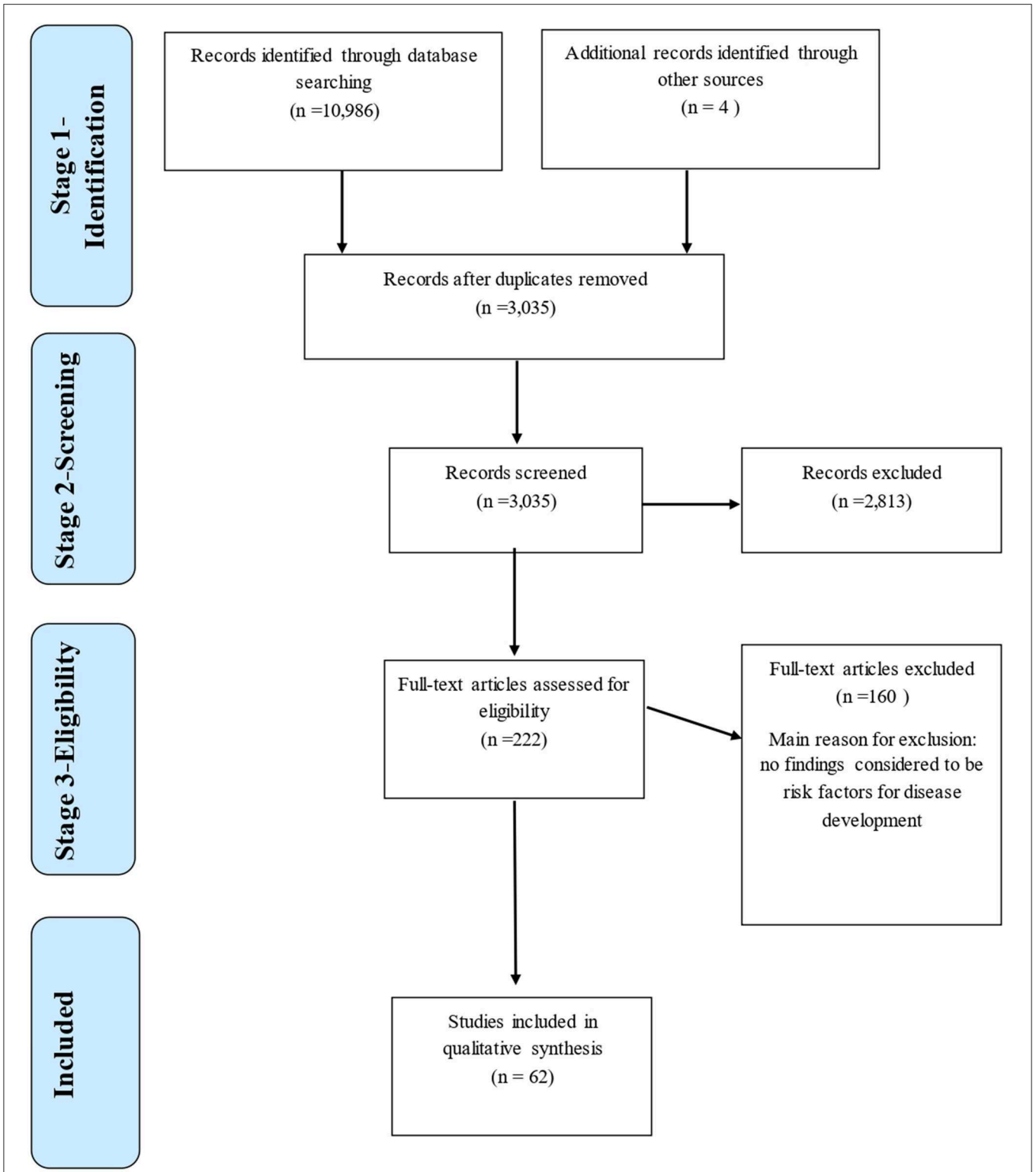

FIGURE 1 | Flowchart adapted from PRISMA Guidelines, 2009 (18) of the literature search strategy used to identify articles with information on risk factors for canine osteoarthritis and its predisposing conditions, with 62 studies retained for further quality evaluation.

TABLE 1 | Search terms used for systematic review literature search (156 combinations in total) conducted on Web of Science and PubMed to obtain literature surrounding risk factors for canine osteoarthritis and its predisposing conditions.

Species	AND	Disease	AND	Keywords
Dog		Degenerative joint disease		Risk Factor*
OR		OR		OR
Canine		Osteoarth*		Predictor*
		OR		OR
		Dysplas*		Susceptibility
		OR		OR
		Dislocat*		Cause
		OR		OR
		Joint fracture		Prevalence
		OR		OR
		Ununited anconeal process		Incidence
		OR		
		Luxat*		
		OR		
		Cruciate ligament		
		OR		
		Developmental elbow disease		
		OR		
		Fragmented coronoid process		
		OR		
		Osteochondrosis		
		OR		
		Osteochondritis dissecans		

**Asterix used as wildcard symbol allowing for variations and spellings of words that start with the same letters.*

TABLE 2 | Information recorded as evaluation criteria of reporting quality based on recommendations from the Critical Appraisal Skills Program (CASP, UK) (19), used to assess the reporting quality of current published evidence of risk factors for canine osteoarthritis and its predisposing conditions as part of the systematic review.

Area of evaluation	Answer and score awarded	
	Yes	No/Not stated
Is there a clear research question, aim or hypothesis and does the study design suitably answer it with appropriate statistical analysis and results stated (values)?	1	0
Was the study period a suitable time frame?	1	0
Is the study design relevant to answer the study question?	1	0
Is the research applicable to the target population?	1	0
Are there any other explanations for the conclusions discussed? (e.g., other confounding variables, result variability due to methods)	1	0
Does the conclusion fit with other studies?	1	0
Does the study provide the full picture so that it is repeatable?	1	0
Was there use of controls?	1	0
Any bias in patient selection?	0	1 (or Y but acknowledged)
Does the research hold any implications (either positive or negative)?	1	0

The maximum possible score was 10.

Final Corpus

The reference lists of all papers included in the final corpus were checked and citations not already captured in the literature search to date were screened from Stage 2 onwards. Papers within the final corpus were categorized based on the primary disorder of focus. Most papers investigated a single disorder and area of risk; where multiple disorders were reported, each paper was categorized based on which disorder featured most prominently.

Inclusion Criteria

Papers published in peer-reviewed journals were included in the search with no timeframe filter. Included papers were either written in or translated into the English language and no filters were included on country of origin.

Papers were included in the final corpus following the title, abstract and full text check only if they were:

(i) Peer reviewed papers in the English language including the topic of canine/dog osteoarthritis (and synonyms) or a predisposing condition. Whilst papers that reported on other species in addition to dogs were included, only the results related to dogs were included within this review.
(ii) Reporting primary research (literature reviews were excluded);
(iii) Reporting research that applied statistical testing to demonstrate increased risk of disease or demonstrated variation in susceptibility to develop or be diagnosed with osteoarthritis or a predisposing condition, such as (but not limited to) genetic or biomarker studies (due to diverse methodologies used in epidemiologic studies, no types of study design were excluded);
(iv) Inclusion of dogs that had been reported to have osteoarthritis (or synonym) or one of its predisposing conditions, apart from in the case of "healthy" control cases.

Reporting Quality Evaluation (QE)

Eligible full text papers were subject to reporting quality evaluation (QE). The quality appraisal tool was created during this study based on adaptations from the Critical Appraisal Skills Program checklist (19). The tool was adapted to assess reporting paper quality by evaluating the reporting of methodology (including risk of bias) and outcomes/results. QE was scored as high (QE-H): 8–10, medium (QE-M): 4–7, or low (QE-L): 0–3 (**Table 2**).

The following details for each paper in the final corpus were recorded in a Microsoft Excel spreadsheet:

(i) Publishing details: paper title, authors, year of publication, journal name, volume number, and page numbers;
(ii) Study details: disease of focus, study design, statistical analyses (test/s used), overall sample size (total number

of dogs included in study including controls where used), and control sample size (number of control dogs where used), whether sample size/power analysis was calculated and reported in the paper;

(iii) Study outcomes: risk factors identified (qualitatively recorded), and the direction and measurement of the risk (whether it increased or decreased likelihood of osteoarthritis development).

RESULTS

Study Selection

At Stage 1, 10,986 papers were returned by searches in Web of Science and PubMed and stored in Endnote. Once duplications had been removed, 3,033 papers were in the pre-screened corpus, and were exported to Excel for inclusion screening. Following Stage 2 screening, 479 paper titles were retained; During Stage 3, after abstract checks, 220 papers were retained (**Figure 1**). After full texts were checked, a total of 57 papers met the inclusion criteria to be included in the Final Corpus. The Final Corpus totaled 62 papers that met the inclusion criteria for review discussing risk factors associated with joint conditions (57 of these from the database search, 4 known separately to the authors, 1 from reference list searches within included papers).

Inter-observer reliability for percentage agreement of papers obtained in the screening stage between the three independent assessors was calculated at 97% across title checks (582/600; 300 papers were randomly selected each and reviewed by the two additional reviewers).

From the Final Corpus of 62 papers, the main disease of focus (i.e., within the paper title or where multiple diseases were discussed, the primary disease of interest) for 20 (32%) papers was hip or elbow dysplasia, 17 (27%) focused on cruciate ligament disease, 16 (26%) on osteoarthritis, 6 (10%) on patellar luxation, and 3 (5%) on osteochondritis dissecans.

Study Characteristics and Reporting Quality Evaluation

Regarding study design, 31 (50%) studies were retrospective cross-sectional, 16 (26%) were retrospective case-control, 11 (18%) were prospective cohort studies, three (5%) were prospective cross-sectional studies and one (2%) was a retrospective cohort study. The existing literature has a wide timespan with publication dates ranging from 1972 to 2019 (47 years). The majority of papers (53%) were published since 2009, 22 since 2014. For papers published since 2014, the disease most frequently in focus was cruciate ligament disease (eight papers; 36%), followed by hip dysplasia (five papers; 23%), patellar luxation (five papers; 23%), osteoarthritis (three papers; 13%), and osteochondritis dissecans (one paper; 5%) (see **Table 3** for study design for each individual paper).

Five of the 62 papers (8%) reported a sample size (and accompanying calculation) within their study. All sample sizes and whether a calculation was reported for each paper are included in **Table 3**. From the Final Corpus, 34 papers (55%) had high reporting quality and 28 (45%) had medium reporting quality (the quality scores for all papers are included within **Table 3**), with scores ranging between 5 and 10 (the maximum score). The areas where papers most frequently lost points were: they lacked a clear research question, methodology reporting was not detailed enough; and/or there was a risk of bias within the study that was not acknowledged by the authors, for example in sample selection.

Risk Factor Results

Full results summarizing the risk factor findings for each paper included in this review can be found in **Table 3**. Across the corpus of papers, 61 (98%) of the papers discussed at least one risk factor that increased the risk (i.e., predisposition toward) of developing a joint disorder, whilst 19 (31%) papers discussed risk factors associated with a decreased risk (i.e., protection against) of joint disorder development. There were six main risk factors (genetics, breed, conformation, age, sex/neuter status, and body weight) reported across the studies, with many studies suggesting joint disease is a multifactorial disorder (**Table 3**). Other risk factors reported to have an association with disease development included diet/feeding, month of birth and early life factors, exercise levels (particularly when young) and type of exercise, and insurance status (**Table 3**). The most frequently reported risk factor was genetics (discussed as particular "risk" genes and chromosomal regions, and disease heritability). Twenty-one (34%) of the 62 papers in the Final Corpus reported genetics as a risk factor for osteoarthritis, or a predisposing arthropathy.

Direction of Risk

Of the 21 papers that discussed genetics, an increased risk associated with specific genes was reported by 20 of the papers. Genetic factors associated with decreased risk of developing osteoarthritis or a predisposing arthropathy were reported in four papers. Nineteen (30%) papers assessed sex and/or neuter status as a risk, all of which discussed sex (both being male and being female) and neuter status (being neutered) as having an increased risk for joint disorders. Seventeen papers (27%) discussed breed as a risk factor; five of which identified breeds that had a decreased risk, with the remaining describing breeds with increased risk of joint disease. Thirteen (21%) papers assessed body weight all of which should an increase of risk with increasing body weight. Twelve (19%) papers identified age as a risk factor; nine papers found an increased risk of joint disease associated with age (increased risk was recorded in older dogs for osteoarthritis, younger dogs for cruciate ligament disease, and conflictingly both younger and older dogs for hip dysplasia), whilst three papers reported a decreased risk associated with age (decreased risk in younger dogs for cruciate ligament disease, and decreased risk for older dogs for patellar luxation). Finally 10 (16%) papers discussed specific conformational traits associated with either an increased risk of joint disease (9 papers) or a decreased risk (1 paper).

DISCUSSION

Reported Risk Factors

The results of this review suggest six key risk factors associated with canine joint diseases. There is currently no weighting

TABLE 3 | Study reporting quality evaluation results and information recorded for the 62 studies that met the systematic review inclusion criteria for canine osteoarthritis and predisposing conditions risk factors.

References	Risk factor paper findings	Direction of risk	Reporting quality evaluation (QE) category and score	Type of study	Overall sample size	Control sample size	Sample size calculated
Cruciate ligament literature evaluation							
Adams et al. (20)	Females	Increased (OR 2 compared to males)	H-9	R-CC	1,368	1,179	N
	Rottweiler breed	Increased (OR 5 compared to crossbreeds)					
	Obesity	Increased (OR 3.8 compared to healthy weight)					
	Younger dogs	Decreased (OR 0.2 compared to dogs >8)					
Baird et al. (21)	Regions on Chr 3 and 33 (most significant)	Decreased (OR 0.1–0.2)	M-7	R-CC	749	456	N
	Regions on Chr 1 (most significant)	Increased (OR 5.96)					
Baird et al. (22)	Collagen genes significantly associated	Increased	M-7	R-CC	271	172	N
Baker et al. (23)	Multiple genetic loci (~172) contribution	Increased	M-7	R-CS	237	139	Y
	Heritability 0.48						
Baker et al. (24)	Significant loci on ROR2 (Cartilage and bone development)	Increased	M-7	R-CS	222	69	N
	Significant loci on DOCK2 gene (immune cell migration)	Increased					
Clements et al. (25)	Neutered	Increased	H-8	R-CC	17	12	N
	COL5A1 and RPL13A upregulated in	Increased					
	14 genes upregulated in rupture	Increased					
	2 genes down regulated in rupture	Increased					
Duval et al. (26)	Large breeds (9 predisposed)	Increased (OR range 2.15–15.33)	H-10	R-CC	1,005	804	N
	Neutered	Increased					
	Greater body weight	Increased					
Grierson et al. (27)	Rottweilers Golden Retriever	Increased (OR 1.89) Decreased (OR 0.36)	H-9	R-CS	511	N/A	N
	Males	Increased (OR 1.72)					
	Overweight	Increased (OR 1.77)					
Guenego et al. (28)	High tibial anatomical-mechanical axis angle	Increased	H-9	R-CS/CC	274	72	N
Inauen et al. (29)	Lower tibial tuberosity width	Decreased	H-8	R-CS	219	73	N
	Greater body weight	Increased					
	Larger proximal tibial tuberosity angle	Increased					
	Younger	Decreased					
Morris and Lippowitz (30)	Larger tibial plateau angle	Increased	H-8	P-C	87	31	N
Necas et al. (31)	Breeds: Am. Staff terrier, Rottweiler,	Increased	H-9	R-CS	183	N/A	N
	Chow Chow, St Bernard, Bullmastiff	Increased					

(Continued)

TABLE 3 | Continued

References	Risk factor paper findings	Direction of risk	Reporting quality evaluation (QE) category and score	Type of study	Overall sample size	Control sample size	Sample size calculated
	German shorthaired pointer, Boxer	Increased					
	German Shepherds	Decreased					
Pecin et al. (32)	5–8 years	Increased	M-7	R-CS	117	N/A	N
	Mixed breeds and Labradors	Increased					
Taylor-Brown et al. (16)	Neutered females	Increased	H-9	R-CC	2,828	1,875	Y
	>3 years	Increased (OR 2.1)					
	Rottweiler, West Highland Terrier, Golden Retrievers, Yorkshire Terriers, and Staffordshire Bull Terriers	Increased (OR 5.4, 2.5, 1.9, 1.8, respectively)					
	Cocker Spaniels	Decreased (0.4)					
	Increasing body weight	Increased (OR 3.4)					
	Insured	Increased (OR 4.0)					
Townsend et al. (33)	Steep medial tibial plateau midsagittal radius of curvature (m-TPr) angle	Increased	M-7	R-CS	18	18	N
Whitehair et al. (34)	7–10 years	Increased	H-10	R-CC	602,317	591,548	N
	Neutered	Increased					
	Females	Increased					
	Rottweiler, Newfoundland, Staff terrier	Increased					
	Old English Sheepdogs, Basset Hounds, and Dachshunds	Decreased					
	Greater body weight	Increased (>22 kg)					
Wilke et al. (35)	86 markers associated with CCLR traits	Increased	M-6	R-CS	90	N/A	N
	4 associated markers on chr 3, 5, 13, and 24	Increased					
Dysplasia literature evaluation							
Beuing et al. (36)	Males	Increased	H-8	R-CS	2,114	N/A	N
	Heritability estimate 0.28	Increased					
Cardinet et al. (37)	Low Pelvic muscle mass index	Increased	H–8	P-C	82	N/A	N
Choi et al. (38)	High distraction index	Increased	M-5	R-CS	87	N/A	N
	Greater weight	Increased					
	Dogs kept indoors through growth	Increased					
Clements et al. (39)	5 SNPs associated with risk	Increased	M-5	R-CC	647	438	N
	5 SNPs associated with protection	Decreased					
	8 haplotypes as risk (5) or protectors (3)	Increased and Decreased					
Coopman et al. (40)	German Shepherd dog, Golden and Labrador retriever and Bernese Mountain dog (Hip)	Increased (prevalence)	M-6	R-CS	7,506	N/A	N
	Rottweilers, Newfoundland, and Sharpei (elbow)	Increased (prevalence)					
Hou et al. (41)	Boykin Spaniel and St Bernard (Hip)	Increased (Incidence)	H–8	R-CS	895,864	N/A	N
	Siberian Husky and Afghan Hound (Hip)	Decreased (Incidence)					

(Continued)

TABLE 3 | Continued

References	Risk factor paper findings	Direction of risk	Reporting quality evaluation (QE) category and score	Type of study	Overall sample size	Control sample size	Sample size calculated
	Rottweiler (elbow)	Increased (Incidence)					
	Rhodesian Ridgeback (Elbow)	Decreased (Incidence)					
	Males (elbow)	Increased					
	3–5 years old	Increased					
Kealy et al. (42)	Non-limited feeding	Increased	M-7	P-C	48	N/A	N
Krontveit et al. (43)	Born Spring and Summer	Decreased	H-8	P-C	501	N/A	N
	Urban/suburban home (breeder home)	Increased					
	Exercise on soft ground, daily stair use	Increased					
	Off leash exercise (from 0 to 3 months)	Decreased					
Lavrijsen et al. (44)	Bullmastiff, Boxer, and Italian Corso dog most prevalent	Increased (prevalence)	H-9	R-CS	35,046	N/A	N
	Golden Retrievers—Female	Increased (prevalence)					
	Labrador Retriever—Males	Increased (prevalence)					
Lavrijsen et al. (45)	Associated regions on chr 8	Increased	H-9	R-CC	122	NS	N
	Candidate genes LAMA2, LRR1, and COL6A3 (disruption in etiology of hip)	Increased					
Leppanen et al. (46)	Born spring and summer	Decreased	H-8	P-CS	10,335	N/A	N
	Older dogs	Increased					
Loder and Todhunter (47)	Females	Increased (OR 1.05)	H-8	R-CS	921,046	N/A	N
	Born in spring and winter	Increased (OR 1.14 and 1.13)					
	Working dogs	Increased (OR 1.88)					
Oberbauer et al. (48)	Increasing age	Increased	H-9	R-CS	1,331,981	N/A	N
	Heritability 0.57	Increased					
Priester and Mulvihill (49)	Large and giant breeds	Increased (Relative risk 3.6 and 10.2)	H-9	R-CS	1,193	N/A	N
	Small and medium breeds	Decreased (Relative risk 0.2)					
Sallander et al. (50)	Exercise by running after balls/sticks	Increased (OR 2.4)	M-6	R-CC	292	NS	N
	High fat intake/energy from fat	Increased					
	Overfeeding/ High body weight	Increased					
Todhunter et al. (51)	HHIP, DACT2, and WIF1 expression	Decreased	M-6	R-CC	32	8	N
	SPON 1, FBN2, EMILIN3, ACAN, IGF1, CILP2, COL11A1, COL8A1, HAPLN, PLA2F, TNFRSF, TMEM, IGFBP expression	Increased					
Torres de la riva et al. (26)	Early neutered males	Increased	M-7	R-C	1,518	N/A	N
Witsberger et al. (52)	Neutered males	Increased (OR 1.21)	H-8	R-CS	1,243,681	N/A	N
	2 months−1 year and 1–4 years	Increased (OR 1.22 and 1.48)					
	Large and Giant breeds	Increased					
Wood and Lakhani (53)	Born July to October	Decreased	M-7	R-CS	9,657	N/A	N

(Continued)

TABLE 3 | Continued

References	Risk factor paper findings	Direction of risk	Reporting quality evaluation (QE) category and score	Type of study	Overall sample size	Control sample size	Sample size calculated
	Parents with high hip scores (parental genetic effect)	Increased					
Worth et al. (54)	Born Autumn (March and April, New Zealand)	Decreased	H-9	R-CS	5,722	N/A	N
Osteoarthritis literature evaluation							
Anderson et al. (9)	Rottweiler, Dogue de Bordeaux, and Old English Sheepdogs	Increased (OR 3.1, 2.8, and 2.8)	H-8	R-CS and CC	455,557	451,361	Y
	Insured dogs, Neutered dogs	Increased (OR 2.02, 1.8)					
	Increasing age (>3 years) and high body weight	Increased (OR 3.55–53.89 and 2.29)					
Andrysikova et al. (55)	High levels of GAGs	Increase	H-8	R-CC	36	5	N
	Higher GAGs in obese dogs	Increase					
Grondalen and Lingaas (56)	Males	Increased	M-6	P-CS	2,046	N/A	N
	Dogs with at least one parent with osteoarthritis	Increased (Relative risk 1–6)					
Hays et al. (57)	Males (increased hip score and risk of osteoarthritis)	Increased	M-7	P-CS	137	N/A	N
	Additive inheritance						
Hegemann et al. (58)	Synovial 5D4 and TIMP-1 increased (ACLR)	Increased	H-8	R-CC	133	30	N
	Higher serum 5D4 and 10-fold lower serum TIMP-1 levels (FPC)	Increased					
	Synovial 5D4 and TIMP-1 were upregulated in dogs (patella luxation)	Increased					
Kealy et al. (59)	Non-restricted feeding	Increased	H-8	P-C	48	N/A	N
	Greater norberg angle and early joint laxity	Increased					
Kealy et al. (60)	Higher body weight	Increased	H-8	P-C	48	N/A	N
	Non-restricted feeding	Increased					
Maccoux et al. (61)	IL-1b expression in synovial fluid and fat pad	Increased	M-7	R-CC	13	5	N
	IL-6 expression in synovial membrane	Increased					
	Synovial membrane IL-8 expression	Decreased					
	IL-10 gene expression in synovial membrane	Increased					
Mayhew et al. (62)	Caudolateral curvilinear osteophytes present	Increased (7.9 times)	M-7	R-CS	25,968	N/A	N
	High distraction index	Increased					
Powers et al. (63)	Caudolateral curvilinear osteophytes present	Increased (3.7 times)	M-7	P-C	48	N/A	N
	Non-restricted feeding	Increased					
Ramirez-Flores et al. (64)	Females	Increased	M-6	P-C	44	N/A	N
	Body weight >10 kg	Increased					
Runge et al. (65)	Non-restricted feeding	Increased	M-7	P-C	48	N/A	N
Runge et al. (66)	High distraction index	Increased (OR by breed)	H-8	R-CS	4,349	N/A	N

(Continued)

TABLE 3 | Continued

References	Risk factor paper findings	Direction of risk	Reporting quality evaluation (QE) category and score	Type of study	Overall sample size	Control sample size	Sample size calculated
	Higher weight	Increased					
	Older dogs	Increased					
Smith et al. (15)	High distraction index	Increased	H-9	R-CS	15,742	N/A	N
	Weight	Increased					
	German shepherd dogs	Increased (4.95 times)					
	Increasing age	Increased					
Smith et al. (67)	Non-restricted feeding	Increased	H-8	P-C	48	N/A	N
Szabo et al. (68)	Circumferential femoral head osteophytes present	Increased	M-7	P-C	48	N/A	N
Osteochondritis dissecans literature evaluation							
Guthrie and Pidduck (69)	Males	Increased	M-6	R-CS	46	N/A	N
	Multifactorial mode of inheritance						
	Higher heritability in males	Increased					
Ohlerth et al. (70)	Osteophyte formation	Increased	H-8	R-CS	351	N/A	H
Slater et al. (71)	Drinking well-water	Increased	H-10	R-CC	91	60	N
	Playing with other dogs daily	Increased					
	Feeding specialty dry food	Decreased					
	High dietary calcium	Increased					
Patella luxation literature evaluation							
Bound et al. (72)	Small Breeds most prevalent	Increased	H-10	R-CS and CC	155	42	Y
Maeda et al. (73)	Toy Poodles, Pomeranian, Yorkshire Terriers, and Shibas	Increased	M-7	R-CS	2,048	N/A	N
	Genetic- higher risk if littermate has PL	Increased (16.2-fold)					
Nilsson et al. (74)	Heritibality: 0.25 (Chihuahua) 0.21 (Bichon Frise)	Increased	M-6	R-CS	3,095	N/A	N
O'Neill et al. (75)	Small Breeds- Pomeranian, Chihuahua, Yorkshire Terrier, and French Bulldog	Increased (OR 6.5; 5.9; 5.5 and 5.4)	H-9	R-CS	206,482	N/A	Y
	>12 years	Decreased (OR 0.4)					
	Females	Increased (OR 1.3)					
	Neutered	Increased (OR 2.4)					
	Insured	Increased (OR 1.9)					
Srinarang et al. (76)	Significant SNPs in DAG1 gene	Increased	M-7	R-CC	91	30	N
Wangdee et al. (77)	Heritability 0.44	Increased	M-7	R-CS	339	N/A	N
	SNP Chr 13	Increased		+R-CC	96	48	

C, cohort; CC, case-control; CS, cross sectional; H, high; M, medium; N, no; N/A, not applicable; NS, not stated; OR, odds ratio; P, prospective; R, retrospective; Y, yes.

applied to risk factors in the current literature, because there are no quantified and validated estimates of their relative influence, and their relative effect on disease development and severity is largely unknown.

Genetics

Genetics is seemingly the most influential risk factor, with a large number of papers (21/62) discussing genetics having a significant relationship with specific joint diseases. Whether this reflects the importance of this risk factor for joint diseases, or is resultant of research bias is unclear. Following genome-wide studies, many genes have been identified as being either upregulated or downregulated in affected joints compared to healthy joints, often similar to those genes expressed in human joint diseases (25), and a number of chromosomal regions linked with joint diseases have been identified (**Table 3**). In many cases, these genes are related to growth and development (21–23, 35, 45).

Conformation

Ten studies highlighted that joint disease is affected by conformation, particularly relating to body and leg size, and joint angles required by breed standards, inadvertently making some breeds especially predisposed toward and others significantly protected from development of joint disorders (78, 79). There is however limited evidence for the relationship between conformation and genetics, warranting further research in the area. Traits such as low pelvic muscle mass were reported to increase risk of hip dysplasia (37, 38) and osteoarthritis (59, 62), whilst tibial tuberosity width and angle were associated with increased risk for cruciate ligament disease (28, 29). Breeding to reach desired breed conformational appearances and possible inadvertent co-selection of undesirable musculoskeletal conformations can have detrimental effects on welfare (78). Perhaps as a result of high demand for particular breeds, studies have further recorded constantly increasing inbreeding coefficients increasing susceptibility to inherited disorders such as hip and elbow dysplasia (41). Whilst genetics and conformation are non-modifiable factors at the individual dog level, these could be considered modifiable factors when considering future generations of dogs. Therefore, extreme traits and appearances, as well as breeding programmes and practices need to be addressed in order to reduce the number of conformational defects and inherited disorders, if improvements are to be made to canine welfare. Phenotypic selection of breeding stock based on conformational health as well as reduction in inbreeding coefficients have demonstrated reduced prevalence of joint diseases of the hips and elbows (48) and could prove effective as a preventative measure in certain instances (80). However, some research suggests these schemes may not be as effective as hoped, and therefore further strategies for phenotypic and genetic improvements is needed (46).

Breed

Breed was a consistent finding as a common risk factor for joint disease, reported as a risk factor by 17 papers. Certain breeds are discussed as having particular predisposition and risk of joint diseases as a result of both conformation related to breed standards and genetic/heritability components, increasing the likelihood of (but not guaranteeing) the development of joint disease in an individual of that breed compared to other breeds. As a non-modifiable risk factor, this increased risk in susceptibility to joint disease can be used to identify "at risk" individuals by their breed, potentially allowing for earlier diagnoses and treatment. However, it should be noted that in some studies, this increased prevalence may reflect the overall breed popularity and breed prevalence within the dog population [particularly studies that only report prevalence (40, 44) or incidences (41)]. Breeds inclusive of but not limited to Rottweiler, Golden Retriever, and Labrador Retriever were found to have increased risk of cruciate ligament rupture with smaller breeds generally having decreased risk (16, 20, 27, 31, 34). Higher hip and elbow dysplasia prevalence was apparent in larger breeds such as Mastiffs, Boxers, Italian Corso dog, German Shepherds, Golden and Labrador Retrievers, and Bernese Mountain dogs (40, 41, 44, 52) whilst smaller breeds such as Pomeranians, Chihuahua, Yorkshire terrier, and French Bulldog had higher odds of developing patellar luxation compared to crossbreeds (75).

Body Weight

Body weight was another important risk factor associated with joint disease development identified here. In some cases, it was unclear whether body weight reflects mainly breed size or body condition. However higher body weight, and thus an increased load on weight-bearing joints (both larger breed dogs, and overweight individuals) was found associated with an increased risk of disease in all papers that it was reported. Overweight dogs were significantly more likely to develop cruciate ligament disorders, with obesity almost quadrupling the risk (odds ratio (OR) 3.8) (20). Having higher body weight related to size or body condition (no OR reported) increased the risk of developing elbow arthrosis (50).

No significant association between type of diet (such as home-prepared or commercial) and elbow and hip diseases was found; however high fat intake was positively associated with hip and elbow disease (50). Non-restricted feeding during growth and development has also been identified as a risk for developing both hip dysplasia and secondary hip osteoarthritis potentially a result of increased mechanical load in weight bearing joints (42). Furthermore, leptin has been found to be associated with osteoarthritis (81) and is found in higher levels in dogs that are overweight or obese (82), providing a possible alternative mechanism for osteoarthritis development. In studies conducted on paired littermates, one of which was on a control diet and the other on a restricted diet (25% less food than the control), dogs in the control group had an increased body weight and significantly increased development of osteoarthritis, which was also more severe. The onset of osteoarthritis was significantly delayed in the group with restricted intake (83). Therefore, as a modifiable risk factor, this provides evidence that appropriate feeding in order to maintain a lean body condition and therefore improved phenotype should be sustained throughout the dog's life to reduce the risk of joint disease (59).

Sex and Neuter Status

Neutered individuals were significantly more likely to have a joint disease compared to entire individuals in all studies that explored neutering as a risk factor, and therefore robust further study is needed to understand the possible relationship behind this. Associations between neutering and weight gain that have previously been highlighted in the literature (84) could at least in part explain the apparent increased risk of osteoarthritis development in neutered dogs also identified in this review [(16, 20, 25, 26, 34, 52, 85); **Table 3**]. Additionally, the impact of neuter status may be due to changes in gonadal hormones, higher levels of which can indirectly protect the joints and affect growth rates and development (52). It should be noted that for many of these studies the results may be heavily confounded by factors such as age. With neutered dogs most likely to be older on average than entire dogs, they may be at increased risk of development as a result of age rather than neuter status itself (86, 87).

Sex is also discussed as a risk factor in many studies, all reporting an increased risk associated with either being male or female, with some conflicting findings for individual disorders, likely a result of confounding by other factors not taken into consideration. This highlights the need for more comprehensive study of sex as a risk factor, and potential confounding factors and interactions between other risk factors. For example, differences in findings between sexes could also largely be a result of interactions of other confounding factors, such as body size and weight, neuter status, and hormone differences.

Age

There is no way to clearly determine when osteoarthritis or other joint conditions first developed, or when a predisposing disease process has or hasn't progressed into osteoarthritis, making age as a risk factor problematic and laden with assumptions. Many studies discuss aging as a potential risk factor, suggesting joint deterioration occurs increasingly with age and therefore suggesting older age as a risk factor for CCL and osteoarthritis. However, there is conflict in some papers' findings for dysplasia where increased risk was found in both younger and older dogs (**Table 3**). Again, many of these studies neglect to examine other variable interactions that could be involved in this progression deterioration. This conflict may further be a result of the reporting of chronic disease such as joint disorders, with a mix of reporting between prevalence or incidence across studies, and differences in terminologies for disease stage used across the studies. The incidence (new cases) may not be higher in older dogs but the prevalence (all cases within the population) would be expected to be higher in older dogs. Furthermore, although osteoarthritis may begin at any age, it may not be until it is clinically fulminant and reaches a more advanced stage that it is recognized as such. This is of particular concern in papers assessing primary care data (9). Therefore, findings related to age should be interpreted with caution, and methodological approach should be accounted for when assessing reliability of these findings. Longitudinal studies are warranted to explore the relationship between age and disease development more thoroughly.

Other Factors

Other notable risk factors reported by the literature include month of birth and early life factors such exercise levels and type. The link between month of birth and disease development is likely linked to exposure to differing exercise regimes when young. Those born in months that offer more favorable weather for exercise opportunities had increased risk of joint disease development. This is further supported through findings that identify exercise levels and types (such as chasing balls/toys and regularly playing with other dogs), throughout life but particularly when young, are risk factors for joint disease development, due to over-use of and damage to (developing) joints (43, 46, 47, 50, 53, 54, 71).

Limitations of Evaluating Risk Factors for Canine Joint Disease

With conflicting findings such as age and inconclusive findings such as neutering, the limitations of this field of research in general, as well as the differences in aims and methodological approaches by the studies included in this review should be taken into consideration. Studies that investigate incidence (i.e., new cases of disease) are more likely to give more accurate data regarding age than prevalence studies, where age at diagnosis may be less obvious or available (14). Differences between study populations can also complicate comparisons across studies and result in inconsistencies. Referral dog populations (7) are not comparable to general populations as they are a sub-selection from this population, with the referral process potentially introducing selection bias which may lead to exaggerated findings. In studies that use primary-care veterinary data (9, 16, 75), diagnosis of joint diseases can vary greatly between individual veterinarians and veterinary practices. Furthermore, for some clinicians, clinical examination is enough, however others may require advanced imaging to make a diagnosis which can influence timing of diagnosis and therefore reported risk factors may be vastly different amongst studies. There may also be differences in terminology reported within studies causing further limitations, for example what one may call a hip dysplasia case, in reality may well already be clinical hip osteoarthritis, and reported as such by another. The time span across the literature included within this review is very large (1972–2018) and therefore changes for example in breed popularity and breed standards, research methodologies, clinical diagnostics and management, and even core veterinary knowledge over time may result in differences in findings. Finally, attention should be drawn to the number of studies on particular diseases, as well as particular risk factors. Although the corpus of 62 papers identified through the systematic evaluation process includes numerous joint diseases and conditions, the literature is fairly sparse for individual conditions. This relatively low number of papers reflects the need for further research in to risk factors for joint disease. The most frequently reported disease was hip dysplasia (32% of papers) and most frequently reported risk factor was genetics (34% of papers). Whilst this seemingly may imply that hip dysplasia is a high priority in veterinary medicine, and that genetics is the most influential or important risk factor for joint diseases, this could be simply resultant of research bias, and is more reflective of data availability and ease of access to pre-existing data. Further study into joint disease severity and prioritization, as well as risk factor weighting is warranted in order to quantify the influence of risk factors on disease.

Due to the diverse methodologies of epidemiological studies, no exclusions of literature were made based on study design, in order to include all papers that reported an increased risk of disease. As such, the database of papers included within this review is heterogeneous, and therefore it is unsurprising there are conflicting findings between studies, making comparisons limited and conclusions difficult to make. Furthermore, the majority of the studies (77%; 48 out of 62) in the existing literature are retrospective in design (see **Table 3** for further study

detail). As such, they are able to identify risk factors associated with development of osteoarthritis and joint diseases but are fundamentally unable to show causality. An understanding of causality is needed to move toward the development of effective control strategies. The strongest evidence for causality would therefore come from prospective longitudinal cohort studies (methodology adopted by only 23% of papers in this review). However, they also need appropriately calculated sample sizes to robustly identify and quantify risk factors across the lifetime of a dog, taking into account as many confounding factors as possible.

In order to return as many papers possible in the search output, only the Boolean operators "AND" and "OR" were used within search terms and these were searched for in all fields of papers (title, abstract, and full text). No terms were included as "NOT" so as to avoid inadvertently excluding possible references. However, whilst every effort was made to capture all current published papers on the topic of risk factors, it should be noted that some papers may still have been missed using this strategy, for example in the instance where they are not available on the searched databases. Furthermore, only results that focus specifically on canine osteoarthritis were included within this review, and therefore there may be risk factors identified in other canids and species which are not considered within this review. It should also be noted that misinterpretation of papers and reported data included in this review is always a possibility, along with human error in the systematic search, which may result in some literature being missed. As mentioned, limiting the inclusion criteria to articles published in peer-reviewed journals may have led to some level of unavoidable misrepresentation due to publication bias, however the limited availability and reliability of unpublished or non-peer reviewed gray literature makes this exceptionally hard to include. The QE scale used in this paper to evaluate reporting quality was adapted from a pre-existing scale to suit the heterogenous styles of literature, and therefore other factors may also influence the overall quality of these studies. The scores given in this paper are not a final grade but allow for comparison across the studies, and indications for key areas that studies lack in their reporting. In this scale, every point of the evaluation was equally weighted. In reality, certain points may be a greater indicator of quality than others, however, with an absence of evidence to support what this weighting should be, it was most appropriate to attribute equal weighting to all criteria. The evaluation of the papers is at least partly subjective and as such, inter-individual evaluations may differ. Whilst the percentage agreement score was high between the three assessors on this paper, such evaluations cannot be considered truly independent as the assessors were part of the same research team and are perhaps likely to share similar views on relevance.

Reporting Quality and Future Studies

Reporting quality among the final corpus of 62 papers ranged from medium (5–7) to high (8, 9, 39) (**Table 3**), however, there is likely to be publication bias here as one of the requirements of this review is that included papers must have been peer-reviewed. It is therefore unsurprising that the papers are at least of medium quality (5/10). However, looking forward it is important that papers reporting quality and methodological design are of high quality in order to ensure reliability and validity of results and repeatability of studies. The most frequently occurring reasons for papers scoring below QE-H were, (i) that the research question was not clear, (ii) methods were not described fully, such that replication would be difficult, and (iii) potential bias such as sample selection. With these reasons in mind, in order to improve the reporting quality of future studies, it is recommended that a clear research question/hypothesis should be created prior to investigation and also reported in within the paper in a clear and concise manner. Subsequent methodology that appropriately samples the population and answers the research question yielding high quality and valid results is also needed, and sufficient detail should be provided regarded methodology within paper manuscripts. As discussed above, in the instance of risk factor analysis, longitudinal studies that can demonstrate causality would be of benefit to strengthen the current evidence base, and make future study comparisons more robust allowing more reliable conclusions to be drawn from the existing literature. Finally, with regards to reporting quality, only a small number of papers within this review reported sample size calculations (8%). Researchers should ensure these are both conducted and reported within future papers in order to form an appropriate sample population within their study, so that their results and findings that can be extrapolated confidently to the population of interest.

Due to the relatively small number of papers for a common condition in veterinary medicine, further studies are necessary in order to support the current papers' conclusions and extend the current evidence base. Research focus is particularly warranted where inconsistencies and conflicting outcomes have been found across the published studies. Specifically, a deeper understanding of the known risk factors contributing to joint diseases and identification of any as yet unreported factors, as well as the development of genetic screening tests, mapping of significant gene regions, and identifying gene functions would be particularly timely. Prevalence and incidence data of osteoarthritis resulting from predisposing conditions, as well as individual disease prevalence and incidence is currently lacking. There is also a lack of understanding of the nature of the interactions between known and potential risk factors not reported in the published literature. Different risks need to be further explored in order to determine their relative effect on disease development and severity, for example obesity vs. age, and understand their interactions. Finally, further exploration into early detection and diagnosis is needed in order to reduce the number of affected individuals that are bred from, and subsequently develop osteoarthritis.

CONCLUSION

Here, a summary of published literature investigating risk factors for osteoarthritis and its predisposing conditions is presented. Six key risk factors were identified in the published literature, which were a mix of both modifiable and non-modifiable factors. Frequent reference to genetics is

made in current literature highlighting a strong relationship between joint disease and certain genes related to growth and musculoskeletal development, as well as breed and conformational predispositions, highlighting "at risk" individuals. Identifying these individuals may allow for earlier diagnosis and management, and allow implementation of genetic and conformational screening programs to reduce inheritance into subsequent litters. Increasing body weight/condition was also found to have an association with joint disease, most likely due to the increased load on joints. Some identified risk factors such as age and neuter status warrant further investigation to understand more fully their relationship with joint disease, taking into account potentially confounding variables, particularly as there are other health and welfare benefits associated with aspects such as neutering. Other lifestyle risk factors are more easily managed and modifiable, such as the dog being overweight, and therefore preventative methods can be actioned directly. Osteoarthritis continues to be highly prevalent within the dog population, with substantial implications for quality of life and welfare. Understanding the key risk factors for the development of osteoarthritis and conditions that predispose it, is the first step to identifying means of controlling and ultimately reducing it within the population through preventative methods and control strategies. This study highlights these factors, as well as current literature gaps where further high-quality research is warranted.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

KA conducted the systematic review, analyzed the outputs, and wrote and edited the paper. LC was responsible for study design and setting out the systematic review protocol, supported the analysis of outputs, and edited the paper. HZ, DO'N, and RM supported the study design and analysis of outputs, and edited the paper.

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Use of Reticulated Hyaluronic Acid Alone or Associated With Ozone Gas in the Treatment of Osteoarthritis Due to Hip Dysplasia in Dogs

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This study aimed to evaluate reticulated hyaluronic acid alone or associated with ozone gas in the treatment of osteoarthritis due to hip dysplasia in dogs. Fourteen client-owned dogs were randomly assigned into two groups: Group 1—single intra-articular injection of hyaluronic acid; Group 2—single intra-articular infiltration injection of hyaluronic acid associated with ozone gas. Each hip joint received an average of 0.75 mL of reticulated hyaluronic acid ultrasound-guided. Ozone gas at a dose of 45 μg/mL was incorporated into hyaluronic acid by insufflation. Dogs were evaluated for body condition scoring, orthopedic examination and radiographic scores of the hip joints, goniometric measurements of the hip joints, visual gait score, and kinetic analysis. The evaluations were conducted immediately before treatments (M0), and at days 30 (M1), 60 (M2), and 90 (M3) after treatments. There were no significant differences in body mass and body condition scoring (5-point scale) in each group in all evaluation moments. The scores of orthopedic examination of the hip joints showed statistical differences in each group between moments (M0 > M3), but differences were not observed between groups. No statistical differences were found for radiographic scores in each group between moments, but differences were observed between groups immediately prior to treatments (G1 > G2) and 90 (G1 > G2) after treatments. Goniometric measurements of hip flexion and extension showed no significant differences in each group between moments or between groups. No statistical differences between groups were found concerning the lameness score. There were significant differences for lameness score among moments in Group 1, being M0 > M2 and M0 > M3, and Group 2 in which M0 > M1, M0 > M2, and M0 > M3. The mean percentage of change of PVF and VI between M3 and M0 in Group 1 was almost null and in Group 2 was positive, being 31.1 ± 29.4 and 10.6 ± 25.4, respectively. In conclusion, the intra-articular viscosupplementation alone or associated with ozone gas allowed improvement of lameness scores and orthopedic examination score. In Group 2 the association of ozone gas had better results on kinetic analysis.

Keywords: viscosupplementation, articular degeneration, pain, treatment, dysplasia

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INTRODUCTION

Hip dysplasia is an orthopedic disease considered very frequent in dogs even with several control-breeding programmes (1). It is considered a biomechanical disease related to anomalous development of the hip joints that begins after birth and progresses during life, and after the establishment of fibrosis and osteoarthritis may show improvement of the stabilization (2, 3). The main goals of hip dysplasia treatment are to obtain relieve pain and to maintain an adequate gait and weight-bearing, as well as to decrease disease progression (1–4).

Adult dogs with hip joints already affected by osteoarthritis may receive surgical treatment (total hip replacement, hip denervation, or femoral head and neck excision) or non-surgical management (weight control, physiotherapy, environment changes, exercise restriction, medications, supplements, acupuncture, or regenerative medicine), being the choice determined by environmental factors and general healthy, dog's temperament, owner's financial conditions, and presence of comorbidities (2–7).

Some of the medications or supplements used are non-steroidal anti-inflammatory drugs, pain relief medication, antioxidants, and chondroprotective drugs (2, 4, 6). Another treatment modality still little explored in the treatment or prevention of naturally acquired osteoarthritis in dogs is viscosupplementation (8–10). Viscosupplementation refers the intra-articular administration of exogenous hyaluronic acid, or hyaluronic acid derivatives to provide pain relief and improve joint mobility (11–13).

Hyaluronic acid is classified as glycosaminoglycan whose molecules interconnect to make a high viscosity solution (12). In the normal joint, hyaluronic acid is responsible for providing synovial fluid viscosity and elasticity, but its concentration and molecular weight are reduced in joint with osteoarthritis (4, 11, 14).

The decreased viscoelasticity of the synovial fluid increases the susceptibility for the development of injuries due to cartilage overload (15). Some mechanisms have been related to the therapeutic effects of the hyaluronic acid, including anti-inflammatory and anti-nociceptive effects, reestablishment of elastic and viscous properties of the synovial fluid, normalization of synthesis of hyaluronan by synoviocytes (11, 14).

In turn, intra-articular ozone has been used in human patients with osteoarthritis to reduce pain, relieve of physical disability and stiffness, in order to promote the reduction of joint inflammation and to improve quality of life (16–21). The action mechanism is not fully understood (20), but a hypothesis it that ozone injected into synovial fluid produces form reactive oxygen species and lipid oxidative products (17). Thus, the ozone in osteoarthritis may be responsible for cell metabolism activation, to reduce prostaglandin synthesis and oxidative stress, and to induce antioxidant enzyme synthesis, as well as to augment oxygen supply to tissues, promoting immunomodulatory effect and improving vascularization, among others (17, 20).

Very few studies have investigated the role of viscosupplementation and ozone gas in canine hip dysplasia (10). Therefore, this study aimed to evaluate reticulated hyaluronic acid alone or associated with ozone gas in the treatment of osteoarthritis due to hip dysplasia in dogs. The hypothesis was that gas ozone inclusion induces a better clinical outcome compared to hyaluronic acid alone.

MATERIALS AND METHODS

Dog Selection

This study was approved by the Institutional Ethics Committee for the Use of Animals (n°. 0101/2018—CEUA). A written informed consent form was signed from each dog's owner before the initiation of the study.

Twenty-three adult dogs diagnosed with osteoarthritis due to hip dysplasia were evaluated. Dogs were selected based on clinical signs; general physical examination, orthopedic and neurologic exams; hematological and serum biochemical tests, including for alanine aminotransferase, urea and creatinine; and radiographic evaluation of the hip joints. The inclusion criteria were hip dysplasia dogs exhibiting clinical signs of pain and lameness, and without any history of previous surgery. The exclusion criteria were dogs submitted to any other surgical procedure in the previous 6 months before the study, dogs receiving anti-inflammatory drugs, presence of other musculoskeletal, and/or neurological conditions.

Treatments

The dogs were randomly assigned into two groups: Group 1—single intra-articular injection of hyaluronic acid; Group 2—single intra-articular infiltration injection of hyaluronic acid associated with ozone gas. Each hip joint received an average of 0.75 mL of hyaluronic acid alone[1] (8 mg/grams) or associated with ozone gas. Ozone gas at concentration of 45 μg/mL was incorporated into hyaluronic acid by insufflation using sterile hypodermic needle 21G × 1 1/2″ (0.8 × 40 mm). Ozone was provided by model 0 & L3.0 RM ozone generator[2].

After general anesthesia *(RMT)* with propofol, each dog was positioned in lateral recumbency to perform ultrasound-guided[3] *(FM)* intra-articular injection in the right and left hip joints. In the area of injection, the hair was clipped and site was disinfected with chlorhexidine. The intra-articular injection was done *(JISSJ)* with needle (21G × 1 1/2″) or mandrel of the 20G catheter attached to 1 mL syringe inserted at the midpoint of the proximal edge of the greater trochanter.

Body Condition Scoring (BCS)

A 5-point scale was used to evaluate BCS (22), which were conducted *(JISSJ)* immediately prior to treatments (M0), and at days 30 (M1), 60 (M2) and 90 (M3) after treatments.

[1]Hialurox; São Carlos, São Paulo, Brazil.
[2]Ozone & Life; São José dos Campos, São Paulo, Brazil.
[3]MyLab Alpha, Esaote®; Monções, São Paulo, Brazil.

Hip Examination and Radiographic Evaluation of the Hip Joints

The scores of orthopedic examination of the hip joints *(JISSJ)* based on signs of crepitation and pain on palpation were: 1 - absent, 2 - mild, 3 - moderate, 4 - severe.

Ventrodorsal hip-extended radiographs were performed *(MJM–FM)* under general anesthesia. After 8-h fast, the dogs received pre-medication with acepromazine (0.05 mg/kg) and morphine sulfate (0.5 mg/kg) intramuscularly, followed by anesthetic induction and maintenance with propofol (5 mg/kg, IV). Digital radiographs[4] were done with a 1 m focus-film distance, 60–90 kV, and 5.0–6.4 mAs.

Scoring radiographs for hip dysplasia were based on the Orthopedic Foundation for Animals (OFA) classification (23): 0–normal hip (excellent, good and fair classification), 1–borderline, 2, mild, 3–moderate, and 4–severe. Norberg angle was measured for each hip using a commercial software[5], as previously described (24). All images were stored in Synapse PACS system (Fujifilm) as DICOM-formatted files.

The evaluations were performed immediately prior to treatments (M0) and at day 90 (M3) after treatment.

Goniometric Measurements

The goniometric measurements of the hip joints *(JISSJ)* were carried out using plastic universal goniometer[6], as previously described (25). The dogs were positioned in lateral recumbency and one arm of the goniometer was placed on the axis longitudinal of the femur (greater trochanter to lateral femoral epicondyle of the femur) and other arm on the line sacral tuberosity of the ilium to the ischial tuberosity. Hip joint flexion and extension were determined. The measurements were carried out in triplicate by the same investigator immediately prior to treatments (M0) and at day 90 (M3) after treatments and was selected the median value for statistical analysis.

Lameness Evaluation

Lameness at walk was evaluated (JISSJ) using a visual gait score, based on previously reported (26): 0 (normal use of the limb), 1 (lameness is intermittent), 2 (lameness is evident, but dog shows weight-bearing), 3 (lameness is severe, but dog shows weight-bearing), 4 (intermittent lameness, but the dog did not shows weight-bearing), 5 (the limb is not used). All dogs were filmed during gait analysis.

The evaluations were conducted immediately prior to treatments (M0), and at days 30 (M1), 60 (M2) and 90 (M3) after treatments.

Kinetic Gait Analysis

After acclimatization and familiarization with the environment and pressure-sensitive walkway, each dog was guided to the right of the handler to walk *(JISSJ)* (velocity 0.9–1.1 m/s, acceleration −0.2–0.2 m/s^2) in a straight line over the pressure-sensitive walkway[7]. The system was calibrated as specified by the manufacturer. Approximately 15 trials were obtained for each dog and the first five valid trials were used. Valid trials included those that all four limbs had contact on surface of the pressure-sensitive walkway with the dog maintaining the head in an adequate position during walking. The acquisition and analysis of the data were done using a specific software[8]. The Peak Vertical Force (PVF) and Vertical Impulse (VI) were normalized according to dog's body weight and represented as a percentage of body weight (%BW). The percentage change of the PVF (%BW) and the VI (%BW × s) were calculated as previously described (27): $\frac{\left[\left(X2\right)-\left(X1\right)\right]}{\left(X1\right)} \times 100$

The data were collected and analyzed immediately before treatments (M0) and at day 90 (M3) after treatments.

Statistical Analysis

Categorical data of BCS, scores of orthopedic examination, scoring radiographs, and visual gait score were directly converted as treated as continuous variables for statically analysis *(LECSC)* (purposes (28, 29). The variables BCS and visual gait score were evaluated at dog level, and other variables such as scores of orthopedic examination, scoring radiographs, Norberg angle, goniometric measurements, and kinetic variables were evaluated at joint level. All the analyses were carried out using the statistical software SAS, version 9.3. After data were tested for Gaussian distribution using Shapiro-Wilk normality test, non-parametric tests were used; the Mann Whitney U to compare data between Groups 1 and 2, the Wilcoxon Signed Ranks to compare follow-up data of groups M0-M3, and the Kruskal-Wallis test followed by Dunn test to compare lameness data of M0-M1-M2-M3. A $P < 0.05$ was considered significant.

RESULTS

Of 23 dogs evaluated, 14 met the inclusion criteria that were randomly assigned to two groups. Group 1 ($n = 7$) was composed of four males and three females, three neutered, and four entire, average age of 5.9 ± 2.3 years, average body mass of 38.3 ± 13.8 kg, being three crossbreds, two German shepherds, and two Great Danes. Group 2 ($n = 7$) was composed of three males and four females, six neutered, and one entire, average age of 6.4 ± 2.7 years, average body mass of 33.6 ± 12.6 kg, being three German shepherds, two crossbreds, one Labrador retriever, and one Rottweiler. Dogs were numbered from 1 to 7 for Group 1, and 8–14 for Group 2.

In both groups, the dogs showed no signs of complications due to intra-articular injections. No statistical differences were found between groups and in each group among moments for body mass. BCS were not affected by the treatments. In Group 1, 71.43% ($n = 5$) of the dogs had score three and 28.57% ($n = 2$) had score 4. In Group 2, 42.85% had score 3 ($n = 4$) and 57.14% score 4 ($n = 3$).

[4]GE Health, DR-F; Barueri, São Paulo, Brazil.
[5]ClearCanvas Workstation®; Toronto, Canada.
[6]PVC Carci; Carci®, São Paulo, Brazil.
[7]Walkway High Resolution HRV4; Tekscan Inc. South Boston, MA, USA.
[8]Walkway 7.0 software; Tekscan Inc., South Boston, Massachusetts, USA.

TABLE 1 | Body condition scoring (BCS), orthopedic examination score (Orthop), hip radiograph score (X-ray), Norberg Angle (NA), hip extension, hip flexion, percentage change of Peak Vertical Force (PVF) and percentage change of Vertical Impulse (VI) of the hind limbs with osteoarthritis due hip dysplasia, immediately prior to treatment (M0) and at day 90 after (M3) intraarticular injection of hyaluronic acid (Group 1, 7 dogs, 14 hind limbs), or hyaluronic acid associated with ozone gas (Group 2, 7 dogs, 14 hind limbs).

Variables	G1					G2					M-W test *P*-value
	N°	Min	Max	Mean ± SD	Mean rank	N°	Min	Max	Mean ± SD	Mean rank	
BCS	7	3	4	3.29 ± 0.49	7.0	7	3	4	3.43 ± 0.53	8.0	0.32
Orthop score/M0	14	2	4	2.8 ± 0.7[a]	14.5	14	2	4	2.8 ± 0.7[a]	14.5	1
Orthop score/M3	14	2	3	2.4 ± 0.5[b]	14.5	14	2	3	2.4 ± 0.5[b]	14.5	1
X-ray score/M0	14	2	4	3.4 ± 0.8[a]	10.2	14	2	3	2.6 ± 0.5[a]	18.8	0.01
X-ray score/M3	14	2	4	3.4 ± 0.8[a]	10.2	14	2	3	2.6 ± 0.5[a]	18.8	0.01
NA degrees/M0	14	68	122	94.5 ± 19.0[a]	13.3	14	82	115	101.8 ± 9.5[a]	15.7	0.45
NA degrees/M3	14	68	117	93.9 ± 19.0[a]	13.3	14	80	114	101.1 ± 10.4[a]	15.7	0.45
Hip extension (degrees)/M0	14	76	112	96.4 ± 10.5[a]	12.3	14	66	128	103.1 ± 17.0[a]	16.7	0.16
Hip extension (degrees)/M3	14	68	115	97.4 ± 15.1[a]	12.3	14	66	118	103.9 ± 13.9[a]	16.8	0.15
Hip flexion (degrees)/M0	14	42	76	61.1 ± 10.4[a]	12.1	14	44	92	68.6 ± 14.6[a]	16.8	0.14
Hip flexion (degrees)/M0	14	48	74	59.9 ± 10.0[a]	11.6	14	46	94	72.1 ± 16.7[a]	17.4	0.06
PVF change M3-M0 (%)	14	−33.1	26.8	1.3 ± 19.9	10.6	14	−19.3	78.7	31.1 ± 29.4	18.4	0.01
VI change M3-M0 (%)	14	−66.7	32.1	−2.3 ± 25.5	12.6	14	−32.5	44.6	10.6 ± 25.4	16.4	0.25

Key: %-Percentage; [a,b] Variables with different superscripts letters are significantly different in M0 and M3 ($P < 0.05$, Wilcoxon Signed Ranks test); M-W test-Mann-Whitney U test; Max- Maximum; Min-Minimum; N°-Number.

Scores of Orthopedic Examination and Radiographic Evaluation of the Hip Joints

The scores of orthopedic examination of the hip joints showed statistical differences in each group between moments (M0 > M3), but differences were not observed between groups (**Table 1**).

In Group 1, right and left hip joints of each dog had similar radiographic scores, before and after the treatment, being 57.14% had severe classification, 28.57% moderate, and 14.71% mild. In Group 2, right and left hip joints of each dog had similar radiographic scores, before and after the treatment, being 57.14% had moderate classification, and 42.85% mild. No statistical differences were found for radiographic scores in each group between moments, but differences were observed between groups immediately prior to treatments (G1 > G2) and 90 (G1 > G2) after treatments (**Table 1**). No statistical differences were found between groups and in each group between moments for Norberg angle (**Table 1**).

Goniometric Measurements

Goniometric measurements of hip flexion and extension showed no significant differences in each group between moments or between groups (**Table 1**).

Lameness Evaluation

No statistical differences between groups were found concerning the lameness score. There were significant differences for lameness score among moments in Group 1, being M0 > M2 and M0 > M3, and Group 2 in which M0 > M1, M0 > M2, and M0 > M3. There were no significant difference ($P > 0.05$) between moments M0-M1, M1-M2, M1-M3, and M2-M3 in Group, and between M1-M2, M1-M3, and M2-M3 in Group 2.

Kinetic Analysis

The mean percentage of change of PVF in M3 was positive in both groups and in Group 2 was bigger than in Group 1 ($P < 0.05$). The mean change VI between M3 and M0 it was also positive in Group 2 and better than in Group 1 (**Table 1**).

DISCUSSION

The present study compared intra-articular injection of hyaluronic acid alone or associated with ozone gas in dogs with osteoarthritis due to hip dysplasia and did not observe better outcome in dogs that received the association, except for kinetic data.

In both groups, average body mass including standard deviation corresponded to medium to large size dogs; the German shepherd breed was the most represented (36%). In general, hip dysplasia is more prevalent in medium to large breeds, brachycephalic breed, and also dogs with a high proportion of body length to height (3, 7).

The Body Condition Scoring showed that 35.71% ($n = 5$) of the dogs were classified as overweight. The excess of body mass contribute to increase joint stress that may cause cartilage degradation (3). In addition, a correlation between obesity and decreased ability to perform exercise has been observed in dogs with hip dysplasia (30). On the other hand, no statistical differences were found in body mass of the dogs within each of the groups among evaluation moments, thus maintaining the uniformity of the sample. It should be considered that weight reduction alone could improve clinical lameness in overweight dogs with hip dysplasia (31).

In both groups, the right and left hip joints of each dog had similar radiographic scores. Radiographic changes in hip dysplasia often do not correspond to clinical presentation, and approximately 25% of dogs may also have spinal lesions (2, 7). Although there were no significant differences in lameness scores between groups, in the evaluation among moments in each group was observed improvement in lameness score after both treatments with intra-articular viscosupplementation. The hyaluronic acid used in the present study had non-avian origin. Hyaluronic acids produced by bacterial fermentation has lower allergenic potential in comparison those avian origin (11, 12). In the present study, no complications related to the intra-articular injection were observed in both groups. However, adverse effects such as arthralgia, effusion, and heat have been observed in some human patients after intra-articular hyaluronic acid injection in knee (11–13).

The hyaluronic acids have been classified in low molecular weight (0.5–1 $\times$ 10^6Da), intermediate molecular weight (1–1,8 $\times$ 10^6Da), and high molecular weight (6 $\times$ 10^6 Da) (12). However, the hyaluronic acid used in the present study has 2.3 $\times$ 10^6Da, according information of the company. There is controversy about the advantages of the different molecular weights of the hyaluronic acid when used *in vivo* (12). Theoretically, the hyaluronic acid used in the present study has effect for a few months due to its molecular weight, unlike longer-lasting effect products. On the other hand, because is a reticulated hyaluronic acid, repeated applications would not be necessary. There is evidence that cross-linking is responsible to extend the duration of intra-articular of hyaluronic acid (32).

Improvement in scores of orthopedic examination of the hip joints and lameness score in both groups, as well as positive percentage change of the PVF (%BW) in 71.43% hind limbs, but with no changes in the radiographic score, NA or goniometric assessment, suggested a positive effect of viscosupplementation to provide pain-relief. On the other hand, a total of 28.57% of the hind limbs had a negative percentage change of the PVF, suggesting a worse function of theses hind limbs (27) despite of the treatments. In a study in dogs with osteoarthritis related to hip dysplasia, lower pain scores and improved clinical signs were observed with a single intra-articular injection of hyaluronic acid (molecular weight 500–730 kDa) compared to intra-articular saline injection in combination with oral nutraceutical and carprofen (10). Also, in a study with dogs with arthritis in one joint (shoulder, elbow, carpus, stifle and tarsus) that were treated by two intra-articular injections of high molecular weight sodium hyaluronate (Hylartil−4.000.000), applied at 3 week interval, or carprofen orally, was found that at 6 weeks the sodium hyaluronate group was significantly better (58% fully recovered and 10% without improvement) compared anti-inflammatory group (8). In addition, in dogs with patellar luxation treated surgically that received sodium hyaluronate (molecular weight 500–730 730 kDa) injected intra-articularly at the time of the procedure, or at the time of the procedure and 1 week postoperative, had improved clinical scores in comparison to control group at the evaluation 4 weeks after surgery (9).

On the other hand, in studies with experimental induced cranial cruciate ligament rupture, no improvement was detected with the use of intra-articular hyaluronic acid (33, 34). In human patients there is also much controversy concerning intra-articular viscosupplementation, with studies showing positive results after administration (11, 35), while other studies have found no benefit (36). The great variety in preparations, number, and technique of applications and heterogeneity of osteoarthrosis cases may contribute for the different results (13, 37). These types of differences also occurs in clinical (8–10) and experimental studies in dogs (14, 33, 34, 37, 38).

Regarding to Group 2 (hyaluronic acid associated with ozone gas), the ozone concentration was 45 μg/mL. In human patients, concentrations from 20 to 30 μg/mL have shown a positive effect of the ozone therapy in the treatment of osteoarthritis, but the studies show lack of procedure standardization (18–20). In general, ozone alone has been administered 1–3 times per week, for 4–6 consecutive weeks or more (16–20). Since in the present study the gas was combined with hyaluronic acid, a single application was used.

The percent changes of the PVF (%BW) and VI (%BW $\times$ s) were statically significant in favor of the Group 2 compared with Group 1, which indicates a positive increase compared to baseline (27). In general, the PVF (largest force) and VI (area under the force-time curve) are decreased during lameness (27), suggesting that intra-articular ozone may have contributed in reducing the pain (14, 16, 19). However, should be considered that despite randomization, radiographic scores were higher in G1 than G2. On the other hand, the radiographic findings did not influenced the scores of orthopedic examination or lameness score. Thus, the absence of difference between two groups for other parameters had suggested that a single application of ozone was not able to avoid radiographic progression of osteoarthritis and improvement in hip extension and flexion. In a comparative study in human patients with knee osteoarthrosis, the group that received intra-articular injection of hyaluronic acid in combination with oxygen ozone showed better outcome than hyaluronic acid or ozone administered separately, but the applications were once a week for five consecutive weeks (39). Thus, further studies are necessary to clarify, including an ozone group, which may considered one of the limitations of the present study. Because intra-articular route in dogs generally requires sedation and/or anesthesia, one option would be rectal insufflation, as used in human patients with rheumatoid arthritis (40).

Another limitation of this study was the use of heterogeneous groups of dogs, which makes difficult the kinetic evaluation (41). In addition, the dogs were evaluated walking, because due the disease the dog may be unable to trot or have difficult to gait trial repetition (41), despite of trotting gait be considered more sensitive than walking gait to lameness detection (42). Thus, to avoid these influences future studies using dogs of the same breed and with the same hip scoring should be considered.

CONCLUSION

In conclusion, the intra-articular viscosupplementation alone or associated with ozone gas allowed improvement of lameness

scores and orthopedic examination score, but on Group 2 the association of ozone gas allowed better kinetic results.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

This study was approved by the Ethics Committee for the Use of Animals (no. 0101/2018—CEUA) of the School of Veterinary Medicine and Animal Science—São Paulo State University (UNESP), Botucatu, Brazil. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

JS, SR, IS, and DM contributed to conception and design of the study. FM and MM performed ultrasound and analyzed the radiographs. RT was responsible for anesthesia. LC done the statistical analysis. JS and SR wrote the original draft of this manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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MINI REVIEW
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Swedish Experiences From 60 Years of Screening and Breeding Programs for Hip Dysplasia—Research, Success, and Challenges

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A screening program for hip dysplasia (HD) was introduced in Sweden during the 1950s for German shepherd dogs, before for a few breeds and now any breed. Degree of canine HD was originally graded 1–4 (slight, mild, moderate, and severe) and used in Swedish screening program up to year 2000 and was thereafter replaced by letters A–E with A and B for no signs/near normal, C for mild, D for moderate, and E for severe HD. Final scoring is based on "the worst" side. In Sweden, 70% of all dogs are registered by the Swedish Kennel Club, and in relevant breeds, almost all breeding stock and 30–50% of all dogs are screened for HD. By an extensive database of all dogs registered since 1976 and mandatory identification by microchip, all results can be linked to dogs well-defined by identity and ancestral background. An implementation of structured screening and genetic health programs resulted in markedly decreased prevalence of HD already during the 1980s. The programs are based on open registries and on positive as well as negative results for identified individuals linked to their ancestral background. The successful decrease in moderate and severe HDs is illustrated for seven common breeds. However, there is also the challenge of a further decrease when already almost all breeding is performed with unaffected breeding stock. Handling that and the increased relative prevalence of less severe grades of HD (grade C) calls for breed-specific breeding strategies, taking into account the prevalence and clinical significance in each breed. Further decrease might rather be achieved by using estimated breeding values and genomic selection instead of more extensive and costly screening procedures. For the public perception of HD, the value of a clear distinction between grades D and E as a good predictor of the clinical entity vs. grade C as a tool to refine the selection criteria for breeding stock is indicated.

Keywords: hip dysplasia, research, screening, breeding, prevalence

As one of the first countries to notice the clinical significance of hip dysplasia (HD) as a developmental disorder resulting in arthritis, active research, and actions to reduce its prevalence have now been performed in Sweden for more than 60 years.

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DURING THE 1950S AND 1960S

Starting to Screen

Although described already in the 1930s (1), HD as a clinical entity of significance was not recognized more widely until the 1950s. Extensive research was then initiated by radiologists and geneticists from the Royal Veterinary College in Stockholm on German shepherd dogs born and raised at the breeding colony of the Swedish Armed Forces in Sollefteå (2, 3).

Although the growing puppies were repeatedly radiographically screened, primarily to predict the clinical outcome, it was soon found that a standardized screening procedure also could be used for selection of breeding stock. Since then, almost all radiological screening programs for canine HD worldwide are based on variations of that procedure. Formal screening also of privately owned dogs was organized by the Swedish Kennel Club in collaboration with the Royal Veterinary College in Stockholm in 1958 (4).

The concept of Norberg angle as an objective measure of the fit between the femoral head and the acetabulum was introduced during the early 1960s by Prof. Sten-Erik Olson and one of his Ph.D. students—Ingmar Norberg (5). Sten-Erik Olson was a real frontier in veterinary medicine and diagnostic imaging with leading work on HD as well as on osteochondroses/elbow dysplasia (ED) (**Figure 1**). Ingmar Norberg was a hip panelist at that time, but then never completed his thesis and, instead, went into and is still applying his practice successfully with horses. The concept of Norberg angle has been widely applied and also criticized, but unfortunately was never formally described.

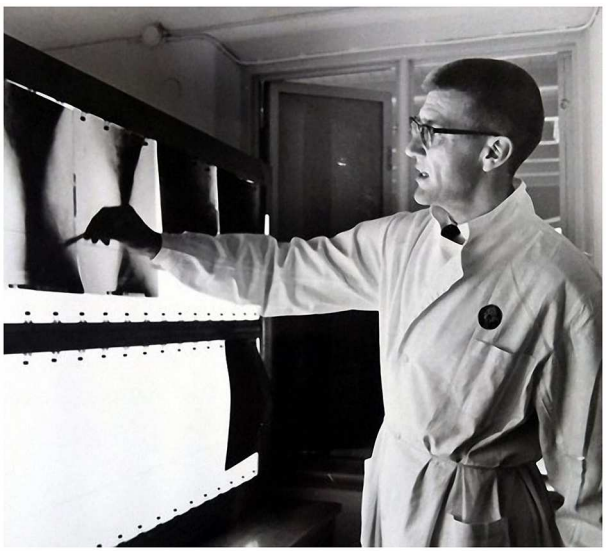

FIGURE 1 | Sten-Erik Olson—a real frontier in veterinary science and diagnostic imaging with pioneering work on hip dysplasia as well as osteochondroses/elbow dysplasia.

DURING THE 1970S

Nutrition and Follow-Up

Based on experimental studies in Great Danes on the effect of "overnutrition" on prevalence and severity of many skeletal diseases (6), more specific studies on the detrimental effect of excessive food intake on the severity and prevalence of HD were performed in a Ph.D. project on HD by Håkan Kasström, another one of the hip panelists at that time (7).

After revealing the nutritional effects on HD, we returned (in the mid-1970s) to the Armed Forces breeding colony to explore possible changes in feeding regimes to reduce the prevalence of HD. A reduced feeding intake was instituted, but the most important finding was that despite extensive screening, the hip scoring result was not taken into account in the selection of breeding stock. An article *Hip Dysplasia and Mentality—Inheritance or Environmental* was therefore published in Sweden in 1976 (8).

In a follow-up study published in 1979 on the result of implemented breeding policies at the Armed Forces Breeding colony, we found very little selection pressure and a decrease in the prevalence of HD during the early 1960s, but there was a dramatic effect in 1973—by selecting not only the status of the Sire and the Dam but also the grandparents and littermates (9).

Base on the material in that study that included all dogs in 401 L born at the Centre from 1965 through 1973, the heritability of HD in that breeding colony was shown to be about 0.4–0.5. The results of this study were actually an early indication of the importance of estimation of breeding values—by including results also from relatives in the selection of a breeding stock for HD—which is in place almost 40 years later (10).

DURING THE 1980S AND 1990S

Cost Benefit

The formal implementation of a screening and health program during the late 1970s and early 1980s for HD in many breeds, and the same somewhat later for ED in some breeds, led to a significant decrease in the prevalence and severity of HD as well as ED with a positive cost benefit also in the general dog population.

Within a Ph.D. project by Lennart Swenson, a former genetic consultant to the Swedish Kennel Club, the effects of selective breeding and its economic value for the HD program operated by the Swedish Kennel Club was investigated based on 83,229 dogs from seven breeds registered by the Swedish Kennel Club born in the years 1976–1988.

A decreasing prevalence of HD, as a result of selection of breeding stock and high heritability, was found and economic analyses showed that the costs of screening and registration of coxofemoral joints were less than the value of dogs estimated to have been saved from moderate, severe, or very severe HD in six of the breeds.

It was concluded that in screening and control programs, based on an open registry with access to family records, a cost-effective decrease in the prevalence of HD can be expected and

is related to the selection of the breeding stock (11). The same positive effect was also proven for elbow arthrosis (12).

Since 1986, there have now been three levels of formal genetic health programs in Sweden for all dogs to be bred in a particular breed:

1) Voluntary screening with central recording of results in open registries freely available on a public website.
2) Sire and Dam are required to have a screening result registered before breeding.
3) Sire and Dam are required to have a screening result A or B (normal hips) before breeding.

In January 2020, 137 breeds were required to have screening results for HD for both parents, out of which 38 breeds also needed both parents to be graded A or B. Results from voluntary screening were recorded in all breeds.

DURING THE 2000S

Clinical Relevance

Within the scope of another Ph.D. project on HD in Sweden by Sofia Malm, a geneticist at the Swedish Kennel Club, the association between grading of hip status assessed by radiographic examination (hip screening) and the subsequent incidence of veterinary care and mortality related to HD, as well as the effects of sedation protocol on screening results, was investigated.

Screening results for hip status from the Swedish Kennel Club and data on veterinary care and mortality from the insurance company Agria were merged based on the registration number of the dog. The study populations of German shepherd, Labrador retriever, Golden retriever, Bernese mountain dog, and Rottweiler included 1,667 up to 10,663 dogs per breed insured for veterinary care and/or life in the years 1994 to 2005.

The effect of hip status at screening was highly significant ($P < 0.001$) for both life and veterinary claims related to HD in all five breeds with an increased hazard ratio (HR) for deteriorating hip status being graded 2–4 [up to year 2000 or later D–E (moderate–severe HD)] as compared with 0 and 1 [up to year 2000 or later A–C (normal hip joints–mild dysplasia)].

The conclusion was that the screening result of grades 2–4/D–E (moderate–severe HD) but not grade 1/C (mild HD) is a good predictor of clinical problems and that selection based on the screening results for hip status can be expected to reduce the risk of HD-related clinical problems (13).

Sedation

To investigate the effect of sedation method on the screening results for HD and ED, a questionnaire survey of routines for hip and elbow screening at Swedish veterinary clinics was related to the results of hip and elbow status for eight breeds (Bernese mountain dog, Boxer, German shepherd dog, Golden retriever, Labrador retriever, Newfoundland, Rottweiler, and St Bernard). A total of 5,877 and 5,406 dogs with a screening result for HD and ED, respectively, were included. The type of chemical restraint used for sedation was shown to have a strong effect on the screening result for HD but not for ED (14). Neuroleptics such as acepromazine was shown to reveal fewer signs of HD than products resulting in heavier sedation.

Following the results of this study, recording of the type of chemical restraint used for sedation during hip screening became mandatory in Sweden. This also made it possible to account for the effect of the sedation method in a model for the estimation of breeding values, EBVs, for HD.

Since 2020, the sedation method when screening for HD has been regulated to not be performed with just neuroleptics such as acepromazine.

Further Studies

During the 2000s, additional Swedish studies have further revealed the effects of diet, weight, and body condition scores (BCSs) as risk factors for HD (15–17).

In ongoing studies, the effects of weight and BCS on health including HD are being further explored (17).

Estimated Breeding Values

Further decreasing the prevalence of HD in populations that are already on mandatory phenotypic screening and even mostly free from any signs of HD calls for more refined selection tools. Estimated breeding values (EBVs) for many breeds have therefore gradually been introduced in Sweden since 2012 (13). Each dog's EBV is calculated by linking pedigree information with data from the registrations of hip status, allowing the genetic risk to be calculated for every individual in the pedigree. EBVs are computerized and updated every week currently (2020-01-01; https://hundar.skk.se/avelsdata/Initial.aspx) for 44 breeds. Also the possibility of combining data for international genetic evaluation has been outlined (10, 18, 19).

International Efforts

Since the 1960s, Nordic hip panelists gather twice yearly to calibrate the procedure and evaluation criteria and have been actively involved in further international standardizations at meetings organized by Fédération Cynologique Internationale (FCI) in 1981 in Dortmund and later in 2007 in Copenhagen.

During the 1990s, great efforts were undertaken by the World Small Animal Veterinary Association (WSAVA) and FCI to harmonize the programs by FCI, Orthopaedic Foundation (OFA), and the British Veterinary Association (BVA)/Kennel Club (KC) (20).

International Hip Panel Meeting in Copenhagen 2021

To harmonize and validate the evaluation and scoring of radiographs, a meeting for actively operating HD panelists will be arranged by the FCI in Copenhagen on September 9–10, 2020. As in former meetings of that kind-—1981 in Dortmund and 2007 in Copenhagen—Swedish and other Nordic panelists will take an active part in planning, running, and following up on it.

Evaluation

In 2019, an extensive evaluation of the Swedish Hip Dysplasia Program was initiated by the board of the Swedish Kennel Club and performed by internal and external reviewers.

The evaluation showed an early successful decrease in all grades of HD followed by a later much slower decrease in affected HD phenotype despite an improved genetic trend.

These findings could be explained by the initial change from usage of unscreened and affected to almost exclusively screened and unaffected breeding stock, and later less selection pressure from phenotypic selection due to less variation (i.e., a larger proportion of dogs scored as normal).

A relative increase in grade C (mild dysplasia) in the later period was partly explained by the shift from the use of less

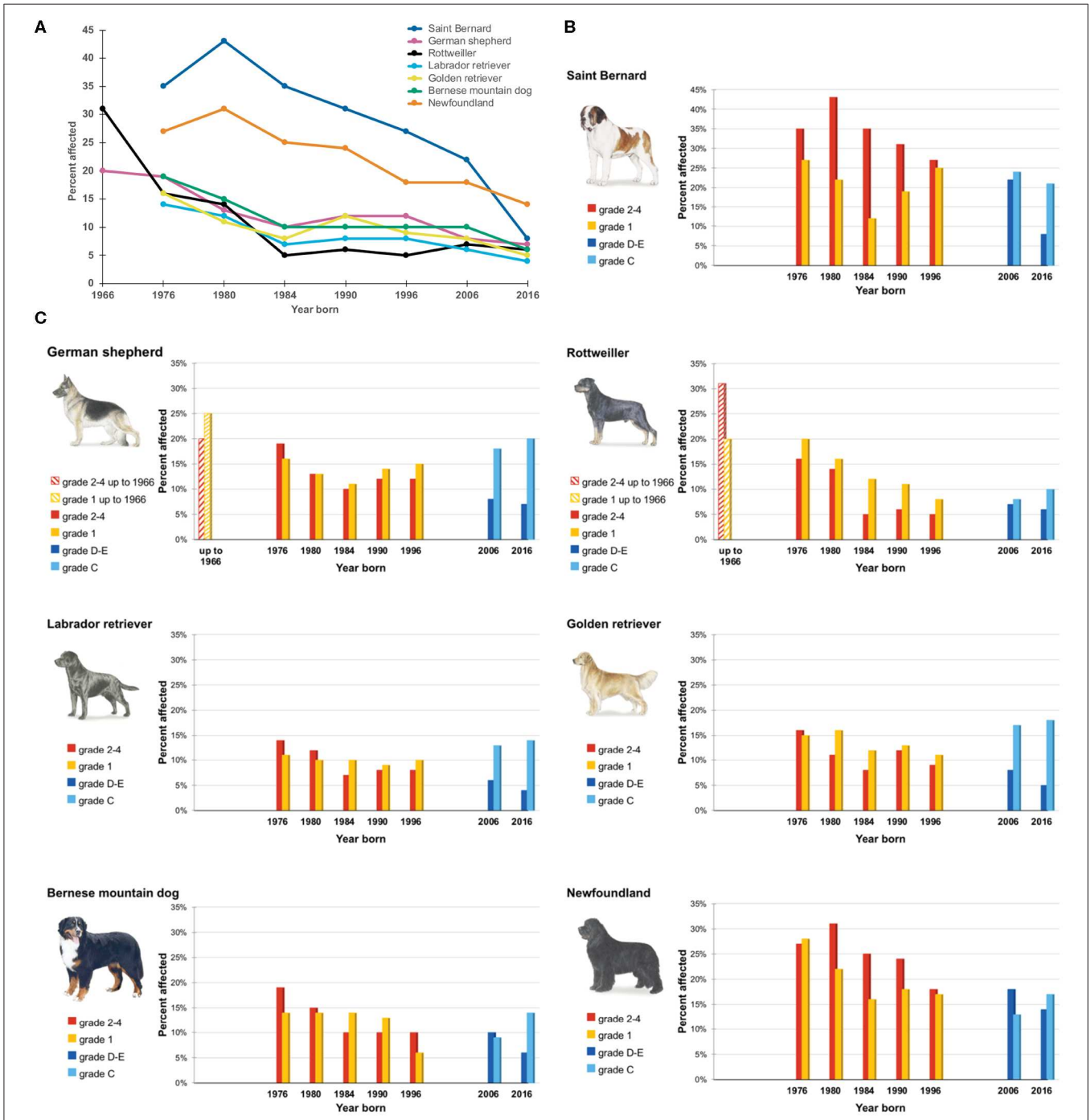

FIGURE 2 | Degree of canine hip dysplasia is graded 1–4 (slight, mild, moderate, and severe) up to year 2000 and thereafter by letters A–E, with **(A,B)** for no signs/near normal, C for mild, D for moderate, and E for severe hip dysplasia. Final scoring is based on "the worst" side. Prevalence of Hip Dysplasia for seven breeds in selected birth cohorts 1976–2016 (since the 1960s for German shepherd and Rottweilers). **(A)** The decline in the percentage of dogs diagnosed with grades 2–4/D–E of HD over the last 50 years for seven breeds. **(B)** Percentage of Saint Bernard dogs diagnosed with different grades of HD over the past 50 years. **(C)** Percentage of dogs from six additional breeds diagnosed with different grades of HD over 50 years (please note the difference in the y-axis scale compared to **(B)**.

to more effective sedation and by the increased use of digitally submitted radiographs.

Based on the findings in the evaluation of sedation, acepromazine as the only preparation is no longer allowed, and only digitally submitted radiographs will be accepted.

Molecular Genetics

The availability of extensive recordings of hip status in Swedish dogs and the use of genome-wide association studies already reported (21, 22) as well as ongoing molecular genetic studies on various phenotypes in the same population have formed the basis also for molecular genetic studies on HD. Recently obtained funding will make it possible to investigate the molecular genetic features of HD in Swedish dogs well-defined by identity and ancestral background.

FURTHER PERSPECTIVES

Having initiated the extensive screening and health programs for HD based on a simple phenotypic screening of individual dogs, it is now a Swedish responsibility to further develop methods to maintain and enable a further decrease in the prevalence of HD. That might rather be achieved by usage of EBVs and possibly also genomic selection instead of more extensive and costly screening procedures. Selection for HD and other health problems is, however, hampered by the fact that these are rarely the prime selection criteria in pedigree dogs.

SUCCESS

The screening programs introduced in Sweden already during the late 1950s made it possible to select screened and unaffected dogs for breeding. That possibility was rewarded and even requested by applied breeding programs from 1984. By using the genetic health programs instituted during the 1980s, a dramatic decrease in the number and fractions of dogs graded as moderate and severe could be achieved. The successful reduction of HD in Swedish dogs since more than 60 years is well-illustrated in **Figure 2**.

By applied selection, the prevalence of moderate to severe HD has been reduced to one third in all of the most commonly predisposed breeds. The data in **Figure 2** are composed of published data on dogs born in the years 1976–1984 (11) and data freely available online for all breeds through the SKK database (https://hundar.skk.se/avelsdata/Initial.aspx). For German shepherd dogs and Rottweilers, data also for the very first dogs screened up to 1966 are available.

Today in Sweden the prevalence of moderate and severe HD (D and E) in most breeds is lower than 10% (German shepherd dogs 7%, Labrador retrievers 4%, Golden retrievers 5%, Bernese mountain dogs 6%, Rottweilers 6%). It is only in a few giant breeds with a few registered dogs and no restrictions on breeding stock that the prevalence of grades D and E is over 10%.

The successful reduction of HD is based on extensive screening mandatory regulated by the Swedish Kennel Club with the results in an open registry including positive as well as negative results and the wide use of this information in the selection of the breeding stock. Since the 1980s, majority of the breeding in most dog breeds are done with both parents screened and free of HD (Grade 0/A or B).

The strength of the results is that the data represent the majority of the dogs of affected breeds in Sweden and that such a high proportion of these are screened for HD. In most predisposed breeds, not only breeding stock but also the majority of other dogs within the breed are screened, adding valuable information for the estimation of breeding values.

The achieved results are to be compared with other breed populations even in countries with long-lasting, but not as extensive, screening programs as in Sweden, e.g., the US, Switzerland, and UK (22–25).

CHALLENGES

In many breeds with generations of breeding stock with normal hip status since the 1980s, it is challenging to achieve a further decrease at the same rate using phenotypic selection.

To achieve further decrease in the number and fractions of severe HD, breed-specific strategies are needed based on the structured usage of estimated breeding values.

A somewhat increased prevalence of dogs with mild HD (grade C) in later years—likely caused by usage of sedation restraints and digital radiology revealing more—has to be taken into account in breed-specific breeding strategies. This is partly accounted for in the prediction of EBVs. In a holistic approach, breed-specific prevalence of HD should be balanced with other health problems within each breed.

There is a value in it and a challenge to influence the public perception of the various grades of HD at screening. A clear distinction between grades D and E as a good predictor of the clinical entity vs. grade C as a tool to refine the selection criteria for breeding stock would reduce the stigma of individual dogs graded C being "diagnosed" as hip dysplastic. It would still stress the value of indicating dogs graded C in the selection of breeding stock.

AUTHOR CONTRIBUTIONS

The author contributed to the conception or design of the work, drafting and revising, and final approval of the version to be published.

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[1]Due to the scope of this review references are mainly restricted to studies on Swedish dogs.

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Hands-Free Conventional Radiographic Ventrodorsal Hip Extended View

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Hip dysplasia (HD) is an important hereditary orthopedic disease in the dog associated with osteoarthritis and inadequate welfare for affected animals. The radiographic ventrodorsal hip extended (VDHE) view is used worldwide to select the better animals for breeding. This view normally is performed with manual restraining of the dog to obtain radiographs with acceptable technical quality. The veterinarian exposition to ionizing radiation is inevitable. In this study, the technical quality of VDHE radiographs and hip measurements was compared in 65 dogs radiographed twice, one with the common veterinarian manual restraining and the other obtained using a hind limb holder device, without the veterinarian within the X-ray room. The variables studied were pelvic tilting, patella displacement index, Norberg angle (NA), and subluxation hip category. The results showed a random distribution of right and left pelvic tilting, patella lateral or medial displacement, and hip subluxation categories in both samples ($P > 0.05$). The holder device positioning showed a better pelvic symmetry ($P < 0.05$) and a similar patellar displacement ($P > 0.05$). The mean ± standard deviation of NA was 101.1° ± 6.2° and 100.9° ± 6.1° in the manual and holder device hind limb restraining, respectively ($P > 0.05$), and the lower limit of 95% confidence interval of intraclass correlation coefficient was >0.75. These results showed statistical reproducibility of NA measurements by the hind limb holder device, and the examiner was protected from exposure to ionizing radiation within the X-ray room.

Keywords: canine hip dysplasia, Norberg angle, reproducibility, hind limb holder, ventrodorsal hip extended view

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INTRODUCTION

Hip dysplasia (HD) is an important hereditary orthopedic disease in the dog associated with osteoarthritis, resulting in an inadequate welfare for affected animals (1, 2). The recommended medical strategy to reduce HD's negative impact on canine populations is to select the better animals for breeding (3, 4). Despite its determinant hereditary component, a genetic test that permits a reliable diagnosis does not yet exist, and it is based on the radiographic examination (3–6). Canine HD phenotype inherence is considered highly complex (7). The radiographic pelvic view must comply with positioning rules to obtain the adequate quality for medical radiographic analysis (1). The conventional ventrodorsal hip extended (VDHE) view is the most used worldwide

(2–4, 8, 9). In this view, the dog is sedated or anesthetized and placed in dorsal recumbency on the X-ray table, and the examiner maintains the dog's hind limbs extended parallel to each other and the stifles internally rotated (8). The medical objective is to obtain a radiograph with symmetrical pelvis and parallel femurs and patella centered on the distal femoral metaphysis (8, 9). Thousands of these types of radiographs are taken daily. The permanence of the examiner within the X-ray room to hold the animal leads inevitably to his exposition to ionizing radiation (8). The interaction of primary X-ray beam with animal origin scatter radiation disperses in random directions in the X-ray room (10). Currently, in England, animal physical restraint in the X-ray room is not allowed unless there is a clinical reason that contraindicates restraint by any other means (11). Thus, the British Veterinary Association has specific recommendations of dog positioning for radiographic hip evaluation (11–13). Precautions must be taken to reduce the possible harmful effects of ionizing radiation to the examiner (10). The ALARA principle "as low as reasonably achievable" for ionizing radiation exposure is a concept in the national and international radiation safety regulations (10).

The main aim of this work was to compare the technical quality of VDHE views obtained using a hind limb holder device fixer with similar views in same animals obtained with the conventional examiner physical restraining. For this purpose, the pelvis symmetry, degree of femoral rotation, Norberg angle (NA), and hip subluxation category (SC) were evaluated. As far as the authors know, there is no published work that has made this comparison; nor is there any similar holder device for the hind limbs to be used for this purpose.

MATERIALS AND METHODS

In this prospective study, 65 dogs (36 females and 29 males) were used from five different Portuguese breeds (28 Portuguese pointer dogs, 27 Estrela mountain dogs, 5 Transmontano cattle dogs, 4 Rafeiro do Alentejo, and 1 Barbado da Terceira). These dogs were presented at the Veterinary Teaching Hospitals of University os Trás-os-Montes and Alto Douro (UTAD) or University Lusófona de Humanidades e Tecnologias in the years of 2018 and 2019 for screening HD. Recorded data included breed, age at time of the radiography, sex, and body weight. The inclusion criteria were dogs older than 4 months, with normal musculoskeletal development in clinical examination, with pairs of VDHE views: one with manual restraining and the other with the hind limbs holder device. Radiographs should have an adequate technical quality for canine HD scoring, with maximum pelvic tilting of 3 degrees and a patellar displacement index from the femoral metaphysis center <0.15 (lateral or medial) (14). The minimum sample size was estimated using a *t*-test table, selecting a statistical significance of 0.05, a medium variable effect size (0.5), and a statistical power of 0.8, and resulted in a sample of 64 observations (15).

All examinations were performed with the dog owner's consent, and all the animal procedures undertaken as part of the work described in this work were performed in compliance with the regulations of our institutions (n° 1044-e-DCV66 2018) and in accordance with the Portuguese and European regulations for animal use and care (European Directive 2010/63/EU and National Decree-Law 113/2013).

Radiographic Procedures

The radiographs were performed, with dogs under deep sedation using medetomidine (Domitor; Orion Corporation, Espoo, Finland) and butorphanol (Torbugesic Injectable; Fort Dodge Veterinaria, Girona, Spain) intravenously. The sedation was reversed with atipamezole hydrochloride (Antisedan; Orion Corporation) intramuscularly. In each animal, two VDHE views in the same sequence were obtained: first the VDHE view with dogs in dorsal recumbency on the X-ray table and the examiner positioning hind limbs in extension and rotated medially (8); and in the second, VDHE view was hands-free where the dog was placed on the X-ray table in a similar position and the rear limbs placed in extension and rotated medially using a holder device (**Figure 1**). This holder device has a rubber groove to fit the dog's tarsus, which was subsequently fixed firmly in each rear limb using a sphygmomanometer with air at 120 mm Hg. Another important component of the positioner is an acrylic stem that is then fixed to the contralateral (we used adhesive strip) to eliminate the supination natural hind limb's force. To complete the limbs fixation under the holder devices, an acrylic base was used coated with a self-adhesive Velcro and on top of everything a cylindrical sandbag of 4 kg (**Figure 1**). The sequence of procedures of this view was as follows: (1) a holder device was fixed firmly on each tarsus of the dog; (2) the examiner put the rear limbs of the dog as if it were to be performed common VDHE view; (3) an assistant placed the acrylic bases under the holder devices; (4) the assistant attached the acrylic stem of the right and left holder devices and placed on top the cylindrical sandbag to maintain rear limbs on medial rotation and extension; (5) the examiner and the assistant left the dog on the X-ray table and went away from the X-ray room.

Radiographic Measurements

The radiographs were obtained in DICOM format using the computed digital radiography Fujifilm FCR Prima reader unit. The pelvic symmetry was evaluated measuring in millimeters the right and left iliac horizontal diameter (IHD) drawing a straight line between the dorsal and ventral iliac cortex at the level of the cranial aspect of sacroiliac joint on right and left sides (16). The IHD asymmetry in millimeters (x) was used to estimate the degrees of pelvic tilting (y) using the regression equation $y = 0.997x + 0.06$ (16).

The horizontal distance between the patellar central vertical axis line to the femoral lateral and medial cortex is used to evaluate if it is centered (same distance to the lateral and medial femoral cortex), external rotated (closer the lateral cortex), or internal rotated (closer the medial cortex) (17). The lateral or medial patellar displacement distance was measured in millimeters from the femoral center, and the respective displacement indices were calculated dividing these distances by the metaphysis thickness (14, 17).

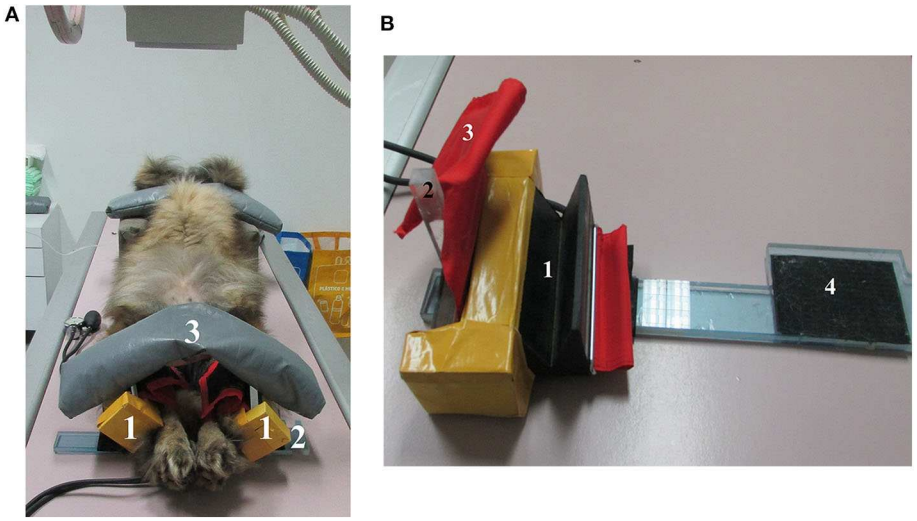

FIGURE 1 | (A) Estrela mountain dog, female, sedated on an X-ray table to obtain the hands-free ventrodorsal hip extended view. (1) Holder device to fix both hind limbs in dog tarsus, (2) acrylic base coated with self-adhesive Velcro, (3) cylindrical sandbag. **(B)** Holder device to fix the dog's tarsus. (1) Rubber groove to accommodate the dog tarsus, (2) acrylic stem that will be attached to the contralateral to maintain hind limb medial rotation, (3) sphygmomanometer to fix the tarsus firmly in rubber groove, (4) acrylic base coated with self-adhesive Velcro to maintain the holder device in position.

The NA was measured in degrees as the angle formed by one line drawn between the centers of the femoral heads and the other from the center of the femoral head to the craniolateral aspect of the acetabular rim (18). The SC was classified from 0 to 6 femoral head evaluating joint congruence and the relationship between the position of femoral head center and the dorsal acetabular edge (13, 14).

The radiographic measurements were performed on randomly chosen digital images of each set; the positioning variables (pelvic tinting and patella displacement index) were measured by J.M., and the HD parameters (NA, SC) by M.M.G., using the software OSIRIS (OSIRIS Imaging Software version 3.1: University Hospital of Geneva, Geneva, Switzerland).

Statistical Analysis

Statistical analysis was performed using the SPSS (SPSS Statistics for Windows version 23.0; IBM Corp., Armonk, NY, USA). Descriptive statistics were performed for all continuous variables. The data analysis was performed at individual joint level.

The χ^2 test of independence was used to determine if there was a significant relationship between the slight right or left pelvic tilting of each set of radiographs, considering pelvis symmetry when the tilting was <1 degree. This test was also used to evaluate the distribution of the slight lateral or medial patella displacement in each set, considering the patella centered when its displacement from the center was <1 mm. The χ^2 test was still used to evaluate the distribution of SCs in each set. The null hypothesis was that there was no relationship between the methodology used in each set of radiographs and the variables distribution (19).

The intraclass correlation coefficient (ICC) and the Bland–Altman analysis were used to study the repeatability of the NA, pelvic tilting, and patellar displacement on examiner and holder device dog's positioning (20). An ICC of 1 indicates perfect agreement, and an ICC of 0 indicates no agreement. A lower limit 95% confidence interval (CI) of ICC >0.75 was defined as an adequate correlation (20). To determine the limits of agreement (LA) according to the Bland–Altman method, we calculated the mean difference ($\overline{d}$) between pairs of measurements and its 95% CI as $\overline{d} \pm 2$ standard error of the mean. When this interval includes zero, measurements are considered to be in agreement (19, 21). Then, 95% LAs were estimated as $\overline{d} \pm 1.96$ standard deviation. Narrower 95% LA is associated with higher agreement between methods. The statistical power was estimated to evaluate the ability of our research design to detect variable differences between groups (15). The $P < 0.05$ was considered to be significant (19, 21).

RESULTS

Sixty-five pairs of VDHE views (130 hip joints) were available from manual-retrained and hands-free holder device view sets (**Figure 2**). The age of dogs ranged from 4 to 93 months [mean ± standard deviation (SD), 24.4 ± 20.2 months], and the body weight ranged from 14 to 68 kg (mean ± SD, 29.9 ± 12.8 kg). The χ^2 test of independence null hypothesis was accepted for pelvic tilting, patellar medial and lateral displacement, and SCs in the comparison between both sets of images (**Table 1**).

In the manual-restraining views, the mean ± SD pelvic tilting, patella displacement index from the center, and NA were 1.4 ± 1.0°, 0.05 ± 0.04, and 101.1 ± 6.2°, and in hands-free holder device views were 0.9 ± 0.9°, 0.05 ± 0.04, and 100.9 ± 6.1°, respectively. The variable paired differences and the statistical power results are described in **Table 2** and **Figure 3**. The ICC for single measures was significant in all cases ($P < 0.05$) with

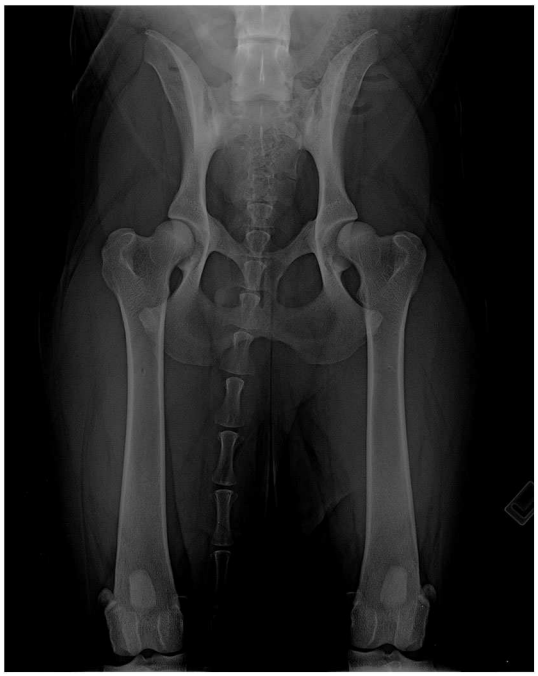

FIGURE 2 | Hands-free ventrodorsal hip extended radiograph of an Estrela mountain dog, female.

TABLE 1 | Pelvic tilting, patella displacement, and subluxation categories in manual-restraining and hands-free holder device.

	Manual restraining	Free-hand holder device	χ^2 test
Pelvis			$P = 0.98$
Symmetry*	34	35	
Tilting to the right	12	12	
Tilting to the left	19	18	
Patella**			$P = 0.44$
Centered	35	36	
Lateral displacement	52	60	
Medial displacement	43	34	
Subluxation category***			$P = 0.80$
1	17	21	
2	68	65	
3	33	35	
4	12	9	

Pelvis symmetry was considered for tilting <1 degree.
Patella centered was considered for lateral or medial patella displacement <1 mm.
***The subluxation categories of 0, 5, and 6 were not used.*

the following values: 0.47 (95% CI, 0.26–0.64), 0.42 (95% CI, 0.27–0.55), and 0.95 (95% CI, 0.92–0.96) for pelvic tilting, patella displacement index, and NA, respectively.

DISCUSSION

Radiography has been used in the diagnosis of HD in dogs worldwide for more than 50 years; there are databases with more than 1 million animals (2, 3, 5, 13). Currently, the main veterinary strategy to reduce the impact on HD in canine populations continues to be based on radiographic diagnosis and breeding selection (3, 4). The main radiographic view used worldwide is the VDHE view, with thousands of these radiographs being taken daily, and in all of them, the dog is positioned by the veterinarian, except in the United Kingdom (2, 3, 13). Here animal physical restraint in the X-ray room is not allowed for HD diagnosis, and some hands-free methodologies based on the use of ropes are available (12, 13). We are advocates of this radiographic approach because we think that whenever possible the ALARA principle in veterinary medicine should be respected. The effect of even low levels of ionizing radiation may accumulate and could represent a potential health hazard (10). However, no study has been able to assess the role of specific low-ionizing radiation exposures in cancer risk (22). A limitation of this study is that the authors do not have practical experience with other hands-free radiographic methodologies; we think that it is important to disseminate these alternative procedures, and we hope in the future to get some free-will followers.

Previous works have shown different levels of longitudinal lateral pelvic tilting and femoral internal or external rotation association to inadequate NA, femoral head subluxation index, or femoral head SC measurements (9, 14, 17, 23). Other showed that longitudinal craniocaudal pelvic tilting does not affect measurement of NA (24). However, the recommended positioning without rotations and according to the standard should be always a fundamental objective in radiographic technique (1, 9), although perfect radiographs are scarce and some level of body rotation is acceptable for HD scoring purposes (9, 14). Normal hips must have good femoral head and acetabular congruence with NA $\geq 105^{\circ}$ and low SC (1, 13). However, other studies argue that the NA cutoff for normal hips should be larger at $\sim 110^{\circ}$, to maximize the specificity of the diagnosis of non-dysplastic hips (25).

The observed slight pelvic tilting and patellar displacement index in our sample is normal and similar to other studies (14). The smaller pelvic tilting ($P < 0.05$) in hands-free holder device views is a positive and desirable effect. The non-equivalent force applied by the examiner on the dogs' left and right hind limbs can be associated to some slight VDHE view asymmetries (26). The similar patellar displacement index in a random distribution not associated with the used method to obtain radiographs is important, as it indicates the good functionality of the use of hands-free VDHE view methodology and having no bias (19). However, the ICC for this variable is low and indicates that there is no true reproducibility (20), but we think that this is perfectly normal and would also happen if this variable was studied in terms of intraobserver variability. One aspect that should be valued is the obtained statistical reproducibility of NA, $P > 0.05$ on paired differences and ICC 95% CI lower

TABLE 2 | Paired variable differences between examiner and hands-free holder device dog's positioning.

Variable	*n*	Paired differences					*P*	Effect size	Power
		Mean	SD	SEM	95% CI				
					Lower	Upper			
Pelvic tilting (°)	65	0.38	0.95	0.12	0.14	0.61	<0.05	0.39	0.88
PDI	130	0.03	0.05	0.00	−0.01	0.01	>0.05	0.05	0.09
Norberg angle (°)	130	0.23	1.96	0.17	−0.11	0.57	>0.05	0.12	0.27

CI, confidence interval; n, number; P, statistical significance; PDI, patellar index displacement; SEM, standard error of the mean; SD, standard deviation.

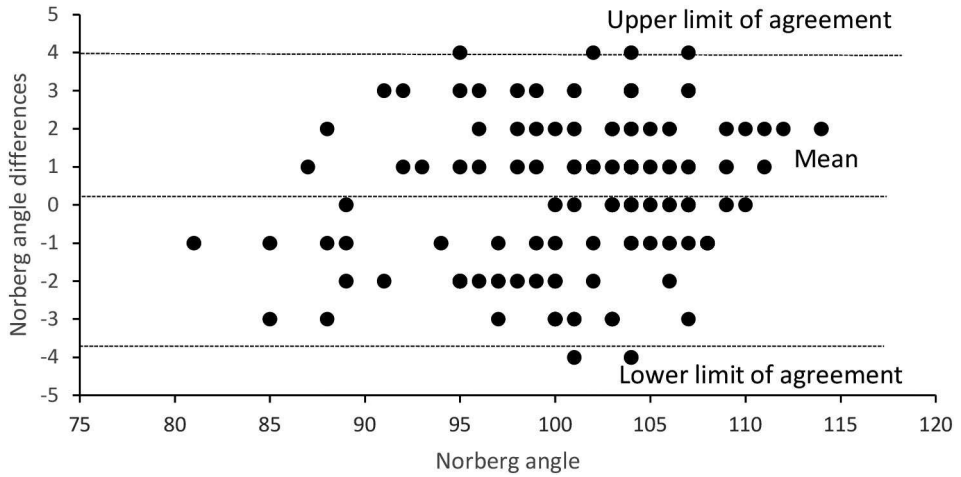

FIGURE 3 | Differences between Norberg angle on examiner restraining radiographic views and on hands-free holder device views plotted against the Norberg angle examiner results.

limit of 0.92, and the equivalent distribution of the SC, variables determinants for the HD grading in the scoring schemes of the Fédération Cynologique Internationale's and the British Veterinary Association/Kennel Club (1, 13, 20). However, the low statistical power of the sample does not allow concluding that there is no significant difference between groups (15). As the size effect is very small (0.12), we will need a sample with approximately 1,000 animals to obtain enough statistical power (0.80) to demonstrate that NA differences are not associated to the used methodologies (15). In medical studies, when the investigated differences are very small, they can be considered with no clinical importance, and it is not worth to detect their origin (27). In the extreme, when the mean of the differences is 0, there is no statistical power that can be used, because the required sample is infinite.

The heterogeneity of the sample associated to the use of medium and large breeds of dogs, different ages, and different examiners should be seen as a positive aspect of the study and methodology, because it allows highlighting all these possible potentialities of the hind limb holder device. However, the small number of breeds and their low representativeness in global terms can be also mentioned as an additional limitation of this study. The hands-free procedure described here is not traumatic for the animal, in contact with its tissues, there is only rubber and the sphygmomanometer, which are both non-traumatic soft materials. The hind limb holder device also does not exert any additional force; it is simply intended to provide adequate stability of the limbs, which are previously positioned by the examiner. In terms of procedure, two people are essential: the examiner to place adequately the dog in VDHE view and an assistant to properly stabilize the holder device. In future studies, it might be interesting to test this holder device and associated technique with different operators, other dog breeds, with and without experience to evaluate the ease of the procedure and the interobserver repeatability, as well as to perform a comparison with the restraint method used in the United Kingdom, the international reference of the hands-free VDHE view.

CONCLUSIONS

This hind limb holder device and associated methodology showed reliability in dog's positioning on the X-ray table to perform the VDHE view, used to evaluate the HD grade. The procedure does not cause any harm to the animal. The holder device allowed obtaining radiographs with better pelvic symmetry and similar patellar displacement. The NA measurements showed statistical reproducibility in comparison

with measurements on a manual-restraining set; however, the study design did not allow obtaining enough statistical power, because of the very small effect size. The use of the holder device allows protecting the examiner from exposure to ionizing radiation within the X-ray room.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The animal study was reviewed and approved by Comissão de Ética—UTAD. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

AS: acquisition of data and drafting of the manuscript. SA-P: acquisition of data and critical revision of manuscript. JM: acquisition of data, critical revision of manuscript, and radiographic measurements. BC: contribution to concept/design and critical revision of manuscript. MG: contribution to concept/design, acquisition of data, data analysis/interpretation, radiographic measurements, and drafting of the manuscript.

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Methods to Improve Joint Genetic Evaluation of Canine Hip Dysplasia Across BVA/KC and FCI Screening Schemes

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The BVA/KC (British Veterinary Association/Kennel Club) and FCI (Fédération Cynologique Internationale) are the main screening schemes used to evaluate the status of canine hip dysplasia (HD) in Europe. Jointly utilizing HD records from both BVA/KC and FCI schemes could improve the reliability of genetic evaluation within and across countries. In this study, HD scores for German shepherd dogs (GSDs) in the UK (using the BVA/KC scheme) and Sweden (using the FCI scheme) were used to investigate how to better operate joint genetic evaluations across the two schemes. It was shown that under a bivariate model, which regarded BVA/KC and FCI scores as different traits, the estimated genetic correlations between the UK and Swedish GSD populations were the same when using BVA/KC total or worse hip scores and for single-country or joint analysis of both the UK and Swedish populations. Under a univariate model that converted BVA/KC scores into FCI scores, the predictability of estimated breeding values was slightly improved by performing a joint analysis.

Keywords: best linear unbiased prediction, estimated breeding value, genetic correlation, genetic evaluation, hip dysplasia

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INTRODUCTION

Canine hip dysplasia (HD) is one of the most common orthopedic disorders in large and giant dog breeds (1). It was reported by the Orthopedic Foundation for Animals (OFA) (2) that 177 breeds were affected by HD, with the prevalence ranging from 0.9 to 75.3%, based on statistics of dogs born between 2011 and 2015. In veterinary practice, HD is diagnosed by radiographic screening and judged by the abnormal characteristics of hip joints. Currently, the BVA/KC (British Veterinary Association/Kennel Club) and FCI (Fédération Cynologique Internationale) are the main screening schemes used to evaluate HD status in Europe (3). Determined by the severity status of HD from normal to severe, aggregated scores of bilateral joints are given by 0–106 (0–53 for each joint) in the BVA/KC scheme and the grade of the worse joint is classified into A, B, C, D, or E in the FCI scheme. In addition to providing a veterinary diagnosis, HD scores/grades can be used to ensure pups are produced from healthy dogs and to calculate estimated breeding values (EBVs) of HD for genetic improvement.

Until now, national genetic evaluations based on HD screening schemes have been implemented in several European countries, (e.g. Sweden, Finland, and the UK). However, with the increasing number of exchanges of breeding animals and semen between European countries (4), joint genetic evaluation across countries should be considered as an approach to the genetic improvement of HD. The availability of EBVs calculated by joint genetic evaluation across countries would encourage and facilitate importation of dogs with high genetic merit. Another potential benefit of performing joint genetic evaluation of HD is increased genetic progress, particularly for countries with small dog populations (5). However, the reliability of joint genetic evaluation has been shown to be limited by genetic connectedness and genetic correlation between countries, especially for countries with different HD screening schemes (6).

In this study, using the German shepherd dog (GSD) as an example breed, BVA/KC scores in the UK and FCI grades in Sweden were utilized to investigate how to better operate joint genetic evaluations across countries with different screening schemes. First, genetic correlations between total or worse hip BVA/KC scores with FCI grades were estimated under a bivariate model (i.e., treating the UK and Swedish scores as two different traits). Secondly, instead of performing a bivariate model across HD schemes, BVA/KC scores for UK dogs were converted from continuous scores into categorical grades to perform a univariate model together with FCI grades for Swedish dogs (i.e., treating the data from the two countries as a single trait).

MATERIALS AND METHODS

Data Preparation

Data used for analysis was provided by kennel clubs from the UK (The Kennel Club) and Sweden (Svenska Kennelklubben), including the pedigree and HD records of GSDs in each country. Dogs that occurred in both UK and Swedish pedigree databases (duplicated IDs) were detected by matching the individual's own and parental IDs. After replacing the duplicates with a unique ID, pedigrees of GSD from the two countries were merged into a combined database containing 877,280 registered animals. If HD was recorded with no screening date, recorded when the dog's age was <12 months or recorded before the year of 2000, then the HD record was removed from data analysis. The HD screening schemes in Sweden changed in 2000 (into FCI grades); thus, for simplicity, we chose to base our study on records since 2000. In total, 17,064 BVA/KC scores from the UK population and 30,909 FCI grades from the Swedish population were used to perform the study. There were no dogs with HD records in both screening schemes. The distribution of BVA/KC scores in the UK GSD population and FCI grades in the Swedish GSD population are shown in **Figure 1**.

For the bivariate model, both total hip scores (HS) and the hip scores for the worse hip (WS) from the BVA/KC scheme were transformed by natural logarithm into transformed total hip scores (THS) and transformed worse hip scores (TWS), as performed for the genetic evaluation of HD in the UK (7):

$$THS = \ln(1 + HS)$$
$$TWS = \ln(1 + WS)$$

Hip grades A, B, C, D, and E of the FCI scheme were converted into scores 1.0, 2.0, 2.5, 3.0, and 3.7 (FCI_{Five}), following the method used for genetic evaluation of HD in Sweden (8). The distribution of transformed BVA/KC scores (i.e., THS and TWS) and converted FCI grades (i.e., FCI_{Five}) used in the bivariate model are shown in **Figure 2**.

For the univariate model, the method of conversion from BVA/KC scores into FCI grades involved transforming WS into the standard FCI five-grade scheme (A, B, C, D, and E) following a suggested conversion originally published by the BVA (0–3 = A, 4–8 = B, 9–18 = C, 19–30 = D, >30 = E) (9). In order to analyze UK and Swedish phenotypes together, BVA/KC scores of UK dogs were converted to FCI grades and then FCI grades of all dogs were converted to scores (as described above), which was defined as trait $FCI_{Five+Five}$. The distributions of $FCI_{Five+Five}$ from the UK and Swedish populations used in the univariate models are shown in **Figure 3**. In addition, a summary of the data used for the bivariate and univariate models is shown in **Table 1**.

Statistical Analysis

The program BLUPF90 (10) was used to run mixed linear models for both bivariate and univariate models using joint UK and Swedish population datasets; variance components were estimated by the average information-restricted maximum likelihood algorithm using pedigree information. The genetic models for bivariate models were formulated as below:

$$\begin{bmatrix} \mathbf{y}_{BVA/KC} \\ \mathbf{y}_{FCI} \end{bmatrix} = \begin{bmatrix} \mathbf{X}_{BVA/KC} & \mathbf{0} \\ \mathbf{0} & \mathbf{X}_{FCI} \end{bmatrix} \begin{bmatrix} \mathbf{b}_{BVA/KC} \\ \mathbf{b}_{FCI} \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_{BVA/KC} & \mathbf{0} \\ \mathbf{0} & \mathbf{Z}_{FCI} \end{bmatrix} \begin{bmatrix} \mathbf{a}_{BVA/KC} \\ \mathbf{a}_{FCI} \end{bmatrix} + \begin{bmatrix} \mathbf{W}_{BVA/KC} & \mathbf{0} \\ \mathbf{0} & \mathbf{W}_{FCI} \end{bmatrix} \begin{bmatrix} \mathbf{u}_{BVA/KC} \\ \mathbf{u}_{FCI} \end{bmatrix} + \begin{bmatrix} \mathbf{e}_{BVA/KC} \\ \mathbf{e}_{FCI} \end{bmatrix} \quad (\textit{Bivariate model})$$

where $\mathbf{y}_{BVA/KC}$ and $\mathbf{y}_{FCI}$ are the vectors of phenotypic values for transformed BVA/KC scores (THS or TWS) and converted FCI grades (FCI_{Five}) in the bivariate models. $\mathbf{X}_{BVA/KC}$ ($\mathbf{X}_{FCI}$), $\mathbf{Z}_{BVA/KC}$ ($\mathbf{Z}_{FCI}$), and $\mathbf{W}_{BVA/KC}$ ($\mathbf{W}_{FCI}$) are incidence matrices, and $\mathbf{b}_{BVA/KC}$ ($\mathbf{b}_{FCI}$), $\mathbf{a}_{BVA/KC}$ ($\mathbf{a}_{FCI}$), and $\mathbf{u}_{BVA/KC}$ ($\mathbf{u}_{FCI}$) are solution vectors for fixed effects, additive genetic effects, and litter effects, respectively. The vectors of fixed effects for both countries consisted of sex, birth year, birth month, and age at screening. In the bivariate model, the vectors of additive genetic effects were assumed to follow a multivariate normal distribution with covariances as $\begin{bmatrix} \mathbf{a}_{BVA/KC} \\ \mathbf{a}_{FCI} \end{bmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix} \mathbf{A}\sigma^2_{a_{BVA/KC}} & \mathbf{A}\sigma_{a_{BVA/KC}a_{FCI}} \\ \mathbf{A}\sigma_{a_{BVA/KC}a_{FCI}} & \mathbf{A}\sigma^2_{a_{FCI}} \end{bmatrix}\right)$, and the vectors of litter effects and residuals were assumed

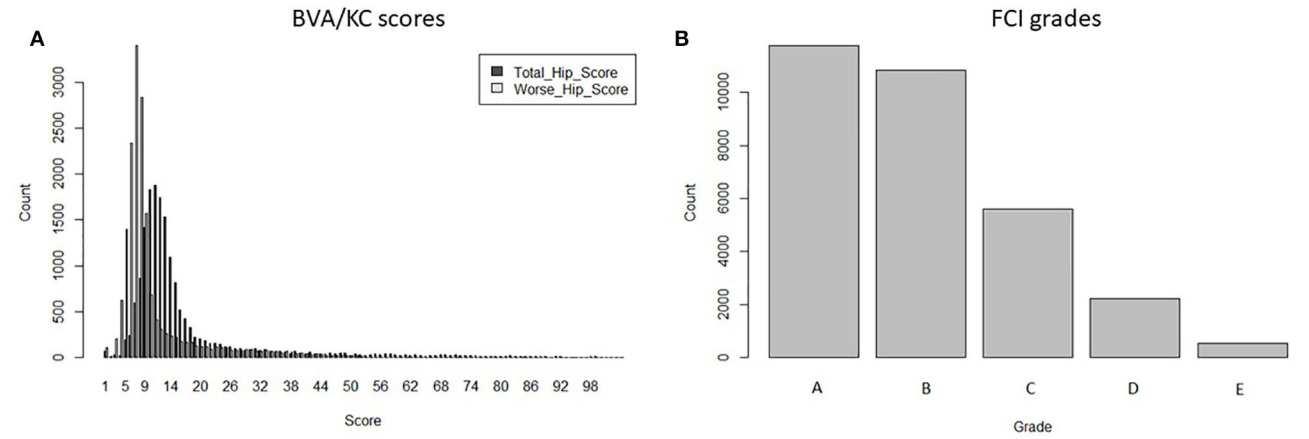

FIGURE 1 | The distribution of hip dysplasia scores/grades recorded in the UK and Swedish German shepherd dog populations since 2000: **(A)** BVA/KC scores by total hip score and worse hip score in the UK; **(B)** FCI grades in Sweden.

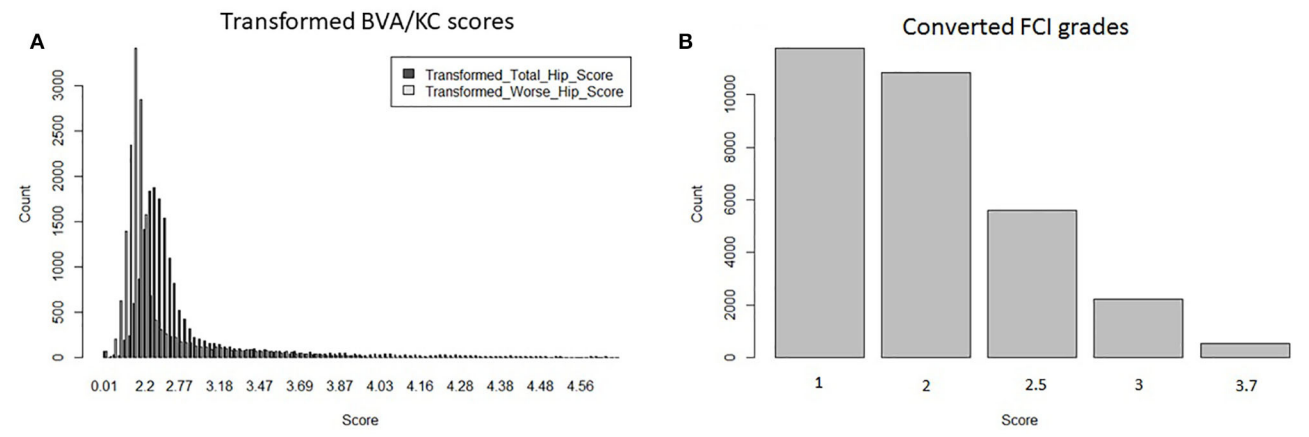

FIGURE 2 | Distributions of transformed hip dysplasia scores/grades used in bivariate models: **(A)** transformed BVA/KC scores by total hip score and worse hip score in the UK population; **(B)** converted FCI grades in the Swedish population.

to follow multivariate normal distributions with no covariances as $\begin{bmatrix} \mathbf{u}_{BVA/KC} \\ \mathbf{u}_{FCI} \end{bmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}\sigma^2_{u_{BVA/KC}} & 0 \\ 0 & \mathbf{I}\sigma^2_{u_{FCI}} \end{bmatrix}\right)$ and $\begin{bmatrix} \mathbf{e}_{BVA/KC} \\ \mathbf{e}_{FCI} \end{bmatrix} \sim N\left(\mathbf{0}, \begin{bmatrix} \mathbf{I}\sigma^2_{e_{BVA/KC}} & 0 \\ 0 & \mathbf{I}\sigma^2_{e_{FCI}} \end{bmatrix}\right)$. The genetic models for univariate models were formulated as below:

$$\mathbf{y}_{FCI} = \mathbf{X}_{FCI}\mathbf{b}_{FCI} + \mathbf{Z}_{FCI}\mathbf{a}_{FCI} + \mathbf{W}_{FCI}\mathbf{u}_{FCI} + \mathbf{e}_{FCI}$$
$$(\textit{Univariate model})$$

where $\mathbf{y}_{FCI}$ is the vector of combined HD phenotypes for UK and Swedish dogs ($FCI_{Five+Five}$). $\mathbf{X}_{FCI}$, $\mathbf{Z}_{FCI}$, and $\mathbf{W}_{FCI}$ ($\mathbf{b}_{FCI}$, $\mathbf{a}_{FCI}$, and $\mathbf{u}_{FCI}$) are incidence matrices (solution vectors) for fixed effects, additive genetic effects, and litter effects in the univariate model, respectively. The vector of fixed effects in the univariate models consisted of sex, birth year, birth month, and age at screening, with an additional fixed effect, country, compared to the bivariate models. In the univariate model, the vector of additive genetic effects was distributed as $\mathbf{a}_{FCI} \sim N\left(\mathbf{0}, \mathbf{A}\sigma^2_{a_{FCI}}\right)$, whereas litter effects and residuals were assumed to follow independent normal distributions $\mathbf{u}_{FCI} \sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_{u_{FCI}}\right)$ and $\mathbf{e}_{FCI} \sim N\left(\mathbf{0}, \mathbf{I}\sigma^2_{e_{FCI}}\right)$, respectively.

Following the variance components estimation described above, heritabilities and genetic correlation (for the bivariate model) were calculated. For each trait/model, the Pearson correlation between EBVs and corresponding phenotypes was calculated to measure the predictability of EBVs, where THS was the corresponding phenotype for the EBV of THS (bivariate and univariate models), TWS was the corresponding phenotype for the EBV of TWS (bivariate model and univariate models), and FCI_{Five} was the corresponding phenotype for

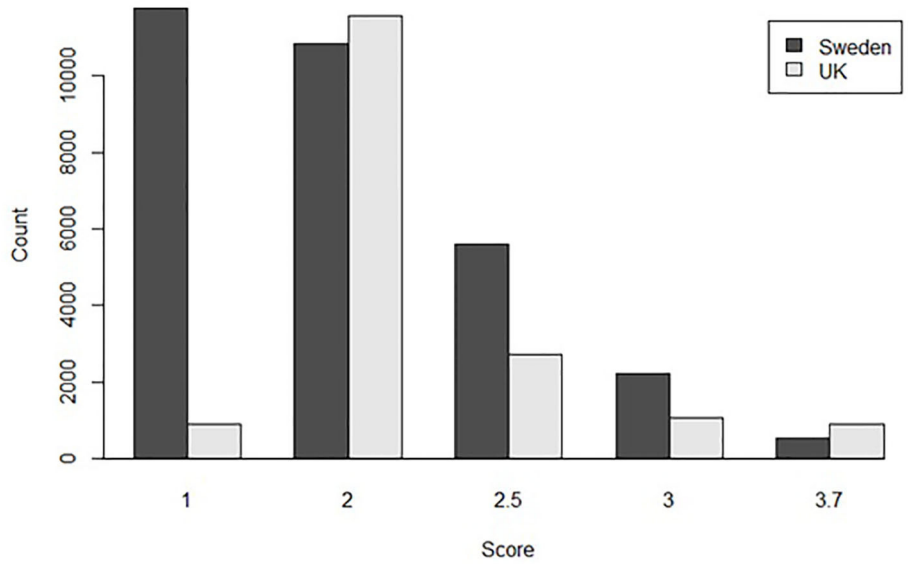

FIGURE 3 | The distribution of converted hip dysplasia scores based on FCI five-level grades from the UK and Swedish populations used together in the combined univariate model.

TABLE 1 | Hip dysplasia (HD) score/grades before and after data transformation/conversion in the UK and Sweden.

Country	Screening scheme	Nr. of records	HD score/grades*	After transformation/conversion*	
				Bivariate model	Univariate model
UK	BVA/KC	17,135	HS (0–106)	THS (0–4.67)	-
			WS (0–53)	TWS (0–2.67)	$FCI_{Five+Five}$ (1.0/2.0/2.5/3.0/3.7)
Sweden	FCI	30,950	A/B/C/D/E	FCI_{Five} (1.0/2.0/2.5/3.0/3.7)	$FCI_{Five+Five}$ (1.0/2.0/2.5/3.0/3.7)

**HS, total hip score; WS, worse hip score; THS, transformed total hip score; TWS, transformed worse hip score; FCI_{Five}, converted FCI five-level worse hip scores; $FCI_{Five+Five}$, converted FCI five-level/five-level worse hip scores for UK/Swedish dogs.*

TABLE 2 | Descriptions of data analyzed in bivariate and univariate models.

Model	Trait*	Number of records	Mean	S.D.	Range
Bivariate analysis	THS	17,064	2.63	0.59	0.00–4.67
	TWS	17,064	2.16	0.57	0.00–3.99
	FCI_{Five}	30,909	1.81	0.71	1.0/2.0/2.5/3.0/3.7
Univariate analysis	$FCI_{Five+Five}$	47,973	1.94	0.68	1.0/2.0/2.5/3.0/3.7

**THS, transformed BVA/KC total hip score; TWS, transformed BVA/KC worse hip score; FCI_{Five}, converted FCI five-level worse hip scores; $FCI_{Five+Five}$, converted FCI five-level/five-level worse hip scores for UK/Swedish dogs.*

the EBVs of FCI_{Five} (bivariate and univariate models) and $FCI_{Five+Five}$ (univariate model). Correlations between EBVs and corresponding phenotypes using single-population datasets were also calculated for THS and TWS in the UK population and for FCI_{Five} separately in the UK and Swedish populations to test whether the predictability was improved through joint-population analysis. Further details of the data used in the analysis are shown in **Table 2**.

RESULTS

Estimation of Heritabilities and Genetic Correlation

In the bivariate models, estimated heritabilities for FCI_{Five} (Swedish dogs) were the same (0.27) whether the corresponding trait for UK dogs was THS or TWS (**Table 3**); the heritability of THS (0.41) was similar to that for TWS (0.39), which resulted from similar estimated genetic variances. The genetic correlation between FCI_{Five} and THS (0.67) was the same as that with TWS (0.67). In the univariate models, the estimated heritability for $FCI_{Five+Five}$ was 0.23, which was lower than the estimate for FCI_{Five} in the bivariate model (0.27).

Correlation Between EBVs and Corresponding Phenotypes

In the UK population, the correlation between EBVs and corresponding phenotypes was slightly higher for THS (0.88) than that for TWS (0.87) in the bivariate models (**Table 4**). The correlations between EBVs and corresponding phenotypes of THS and TWS were the same for the single-population

TABLE 3 | Estimated variance components, heritability, and genetic correlation between BVA/KC and FCI scores (standard error) in the bivariate and univariate analysis.

Model	Trait*	Animal	Litter	Residual	Total	Heritability	Genetic correlation
Bivariate analysis	THS	0.14	0.02	0.18	0.34	0.41	0.67
		(0.01)	(0.00)	(0.01)	(0.02)	(0.02)	(0.21)
	FCI_{Five}	0.14	0.05	0.32	0.50	0.27	
		(0.01)	(0.00)	(0.01)	(0.04)	(0.01)	
	TWS	0.12	0.02	0.17	0.32	0.39	0.67
		(0.01)	(0.00)	(0.01)	(0.02)	(0.02)	(0.22)
	FCI_{Five}	0.14	0.05	0.32	0.50	0.27	
		(0.01)	(0.00)	(0.01)	(0.02)	(0.01)	
Univariate analysis	$FCI_{Five+Five}$	0.10	0.03	0.29	0.42	0.23	-
		(0.00)	(0.00)	(0.00)	(0.01)	(0.01)	

**THS, transformed BVA/KC total hip score; TWS, transformed BVA/KC worse hip score; FCI_{Five}, converted FCI five-level worse hip scores; $FCI_{Five+Five}$, converted FCI five-level/five-level worse hip scores for UK/Swedish dogs.*

TABLE 4 | Correlation between EBVs and corresponding phenotypes in the UK population.

Single-population analysis**		Joint-population analysis**		
Trait*	r(EBV, Pheno)	Model	Trait*	r(EBV, Pheno)
THS	0.88	Bivariate	THS-FCI_{Five}	0.88
TWS	0.87	Bivariate	TWS-FCI_{Five}	0.87
FCI_{Five}	0.94	Univariate	$FCI_{Five+Five}$	0.95

**THS, transformed BVA/KC total hip score; TWS, transformed BVA/KC worse hip score; FCI_{Five}, converted FCI five-level worse hip scores; $FCI_{Five+Five}$, converted FCI five-level/five-level worse hip scores for UK/Swedish dogs; r(EBV, Pheno), the Pearson correlation between EBVs and corresponding phenotypes.*

***Single-population analysis only included UK data; joint-population analysis included both UK and Swedish data.*

TABLE 5 | Correlation between EBVs and corresponding phenotypes in the Swedish population.

Single-population analysis**		Joint-population analysis**		
Trait*	r(EBV, Pheno)	Model	Trait*	r(EBV, Pheno)
FCI_{Five}	0.92	Bivariate	THS-FCI_{Five}	0.92
		Bivariate	TWS-FCI_{Five}	0.92
		Univariate	$FCI_{Five+Five}$	0.94

**THS, transformed BVA/KC total hip score; TWS, transformed BVA/KC worse hip score; FCI_{Five}, converted FCI five-level worse hip scores; $FCI_{Five+Five}$, converted FCI five-level/five-level worse hip scores for UK/Swedish dogs; r(EBV, Pheno), the Pearson correlation between EBVs and corresponding phenotypes.*

***Single-population analysis only included Swedish data; joint-population analysis included both UK and Swedish data.*

analysis (0.88 and 0.87) and joint-population analysis (0.88 and 0.87) using the bivariate model. Using the univariate model, the correlation between EBVs and corresponding phenotypes was slightly increased from 0.94 for single-population analysis (FCI_{Five}) to 0.95 for joint-population analysis ($FCI_{Five+Five}$).

For the Swedish population, the correlation between EBVs and corresponding phenotype, FCI_{Five}, was 0.92 in the single-population analysis (**Table 5**). In the joint-population analysis, the correlations between EBVs and the corresponding phenotype, $FCI_{Five+Five}$, were also 0.92 for both THS and TWS as correlated traits using the bivariate model. The correlation between EBVs and corresponding phenotype of $FCI_{Five+Five}$ was slightly higher (0.94) for the joint-population analysis using the univariate model.

DISCUSSION

By combining the UK and Swedish HD data (including pedigrees and phenotypic records), we investigated two main questions: whether THS or TWS is more appropriate for joint evaluation with FCI grades and whether BVA/KC scores for UK dogs converted to FCI grades are compatible with FCI grades for joint genetic evaluation across screening schemes, so that they can be treated as the same trait in a univariate model.

Heritabilities of HD estimated in this study ranged from 0.23 to 0.41, which were similar to the range previously estimated in the UK and Sweden (5, 7, 8, 11, 12). The estimated heritabilities for THS (0.41) and TWS (0.39) were higher than the reported heritability of THS (0.35) for GSDs in the UK (12). If all HD records (since the 1980's) were used for calculations, the heritability of THS was estimated as 0.35, the same as that previously estimated (12). This is because the total variance of HD recorded since 2000 was lower than the total variance of all HD records in the database, but the genetic variance was similar. In comparison, the heritabilities of FCI_{Five} and $FCI_{Five+Five}$ estimated in this study were 0.27 and 0.23, respectively, which are close to 0.25 as previously estimated for the GSD population in Germany (13), likely due to the similar screening schemes in Sweden and Germany. Differences in the heritabilities for the traits $FCI_{Five+Five}$ and FCI_{Five} reflect differences in the genetic variances for the joint and single populations.

The estimated genetic correlation (0.67) between transformed BVA/KC scores and converted FCI_{Five} grades for the GSD populations in this study was the same as that estimated for

the Golden retriever breed (0.67) but lower than that for the Labrador retriever breed (0.82) when performing a joint genetic evaluation (bivariate model) between the UK and Sweden (5). This difference between breeds may be due to the fact that only HD data recorded from 2000 was used in this study and during this period from 2000 to present only 29 common sires (sires with offspring screened in both populations) existed between the UK and Swedish GSD populations. When the entire dataset of HD records for GSDs since 1980 was used, the number of common sires was 83 and the estimated genetic correlation was 0.80 between the UK and Swedish populations, suggesting that the number of records has a large influence on these estimates. Furthermore, the value of exchanging breeding animals will be greater for higher genetic correlation because the accuracy of EBVs across countries is "discounted" by the genetic correlation (accuracy of EBVs in original countries multiplied by the genetic correlation). Based on results from our previous study (6), very strong genetic correlations (>0.85) are necessary to ensure genetic progress equivalent to selection within an individual country when using foreign sires with EBV rankings in the top 50%, while only moderately high levels of genetic correlation (>0.70) are needed when using foreign sires with high EBV rankings, (e.g. in the top 10%). Based on the bivariate analysis of the UK (THS, TWS) and Swedish data (FCI_{Five}), the correlations between EBVs and corresponding phenotypes for the UK population (THS, TWS) were the same for the single-population analysis (0.88, 0.87) and the joint-population analysis (0.88, 0.87). Similarly for the Swedish population, the correlations between EBVs and corresponding phenotypes of FCI_{Five} estimated from single- and joint-population analyses were both 0.92. The lack of improvement in performing a joint analysis may be due to two factors: (1) the number of phenotypes in each country was sufficient to guarantee high estimation reliability within each population, and (2) there were no dogs with screening records in both countries (i.e., no direct phenotypes were gained from performing a joint-population analysis). After converting BVA/KC scores into FCI grades (UK dogs) and performing genetic evaluation with FCI grades as a common trait (univariate analysis), the predictability in both the UK and Swedish populations was slightly improved by the addition of dogs from the other country, which suggests that there may be a benefit of "borrowing" BVA/KC scores from the UK to implement HD genetic evaluations. This may be particularly useful for countries (unlike Sweden) with few accumulated HD records.

For BVA/KC scores used in the UK population, our results suggest that neither THS nor TWS is a better trait on which to perform joint bivariate analysis with FCI grades. However, for the British national genetic evaluation under a univariate model using BVA/KC scores, the total hip score has previously been suggested to be a more appropriate trait for breeding against HD than the worse hip score due to the presumption that the differences between left and right hips derive from environmental influences rather than genetic effects (7). We only had access to data for the worse hip for the Swedish data in this study, but in the future, if Swedish data can be acquired for both hips, it would be valuable to further investigate this issue.

For both the UK and Sweden, under the joint-population analysis, the univariate model gives a higher correlation between EBVs and the corresponding phenotypes. However, for the UK population, this may in part be an artifact of the non-linear relationship between EBVs (based on natural log-transformed scores) and the original (non-transformed) BVA/KC scores (**Figure S1**), which does not apply to the Swedish data, where the data has not been transformed by logarithm. Furthermore, using the univariate model for both populations, the correlations between EBVs and corresponding phenotypes based on the joint-population analyses were slightly higher than those for single populations, demonstrating that a joint-population analysis would benefit genetic evaluation of HD in both populations.

Joint genetic evaluation across countries has been implemented in dairy cattle since 1983, using a well-defined Multiple Across-Country Evaluation (MACE) model (14), and much higher accuracy has been shown using joint genomic evaluation (15). In order to take an across-country approach for breeding against HD in dogs, the first technical challenge would be the unification of data from different screening schemes. In this study, we demonstrated that converting BVA/KC scores into FCI-like grades, which can then be used as additional phenotypes, could improve the predictability of breeding value estimation in countries using FCI grades (as for the Swedish population).

Recently, a small improvement in accuracy of genomic selection was seen for UK dogs in a joint genomic prediction of Norberg Angle score (one of diagnostic characteristics of HD) between US and UK Labrador retrievers (16); thus, future research could focus on joint genomic selection of HD between BVA/KC and FCI schemes. In addition, a genome-wide association study of canine behavior traits has been performed on a combined dataset of the UK and Swedish GSD populations (17), which suggests the potential to examine genetic factors influencing HD by performing a joint-population association analysis using a univariate model across countries/schemes (e.g., by converting BVA/KC scores in the UK population into FCI grades). This would give the potential to gain further insights into the genetic architecture of HD.

DATA AVAILABILITY STATEMENT

The data is not publically available because the authors don't hold permission to share it. It was provided by The Kennel Club and Svenska Kennelklubben. Requests to access the data should be addressed to these organizations directly.

ETHICS STATEMENT

Radiographs were taken by veterinarians for submission to the British Veterinary Association/Kennel Club

(UK) and Svenska Kennelklubben (Sweden) as part of the canine hip scoring schemes in the respective countries. We received approval for the study from the Veterinary Ethical Review Committee (VERC) and Human Ethical Review Committee (HERC) at the Royal (Dick) School of Veterinary Studies, The University of Edinburgh.

AUTHOR CONTRIBUTIONS

SW, ES, and PW designed the study. SW and JF analyzed the data. All authors contributed to the assembly of the data and preparation of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.00386/full#supplementary-material

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frontiers in Veterinary Science

BRIEF RESEARCH REPORT
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A Regenerative Approach to Canine Osteoarthritis Using Allogeneic, Adipose-Derived Mesenchymal Stem Cells. Safety Results of a Long-Term Follow-Up

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Mesenchymal stem cells (MSC) are emerging as an effective therapeutic tool in treating canine osteoarthritis (OA). In this report, we focused on the questions of whether MSC transplantation has long-term beneficial effects for the improvement in motion and also evaluated the safety of MSC injection. Visceral adipose tissue, a surgical waste obtained during routine ovariectomy served as a source of allogeneic MSCs and used to treat OA. Altogether, fifty-eight dogs were transplanted in the study suffering from OA in the elbow (42 animals), hip (5), knee (8), ankle (2), and hock (1). The effect of MSC transplantation was evaluated by the degree of lameness at a 4-5-years follow-up period based on the owners' subjective observations. The results showed that 83% of the OA patients improved or retained improvement in lameness. Clinical safety of the treatment was assessed by evaluating the coincidence of tumors or other diseases and other adverse reactions (such as local inflammation) after MSC cell therapy. Two incidences of local inflammation for <1 week at the site of injection were reported. No other adverse reactions were detected post-treatment. Sixteen dogs died during the study, 4 due to cancer and 12 due to other diseases, diagnosed by veterinarians. Overall, our survey suggests that MSC transplantation has long-term beneficial effects in reducing lameness. Moreover, no enrichment in a specific cause of death was observed in the transplanted animals, compared to reported literature. Our data suggest that MSC treatment could be an effective and safe long-term therapy for canine OA.

Keywords: dogs, osteoarthritis, mesenchymal stem cell, therapy, safety of therapy, long-term follow-up

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INTRODUCTION

Osteoarthritis (OA), in which the integrity of joint cartilage is disrupted, is one of the most prevalent degenerative diseases, both in dogs and humans (1, 2). Due to the low self-regeneration capacity of the cartilage matrix, the disease is irreversible, thus, the quality of life of the affected animal is expected to decrease gradually (3). In the United Kingdom alone, 200,000 new cases of OA are diagnosed in dogs annually, with both external (injury, obesity, age) and genetic factors

contributing (1, 4, 5). At present, the disease is treated symptomatically by regularly administering non-steroidal anti-inflammatory drugs or by repeated injections of hyaluronic acid (6, 7). Surgical solutions such as arthrodesis or excision arthroplasty (e.g., total joint replacement) are also used in more severe cases (8). However, these are invasive procedures, which may also carry risks of complications such as infections, instability, or periprosthetic fracture (2). In view of the above, the development of a safe and long-term regenerative treatment for OA is highly sought after.

MSCs are excellent candidates for this purpose with a rapidly expanding published literature demonstrating the effectiveness of these cells in tissue regeneration in various diseases (9). An increasing body of evidence now demonstrates that MSCs administered directly into the joint cavity can reduce the chronic pain caused by cartilage degeneration, can induce hyaline cartilage formation, and the treated dogs can live a highly improved quality of life (10, 11). Though MSCs are present in all tissues, adipose tissue is a major source of therapeutic MSCs due to easy access and their high numbers (12).

Besides being able to differentiate into chondrocytes, MSCs produce bioactive molecules, some of which have chondroprotective activity, while others are immunosuppressive and/or anti-inflammatory, thus enabling the safe injection of MSCs into recipients (13). However, the immunological status of the microenvironment is also critical since differentiation of MSCs is inhibited by inflammation (14).

We previously reported that intra-articular injection of allogeneic, adipose-derived MSCs, combined with hyaluronic acid induced a significant improvement in clinical signs of lameness, lasting for 1 year in dogs suffering from elbow OA (11) and now provide data on the long-term (>4 years) health status of the treated animals. To the best of our knowledge, the current report is the first such long-term survey published. We hypothesized that MSCs are effective in reducing lameness and improving the quality of life of animals and that local MSC transplantation is not associated with an increased prevalence of other diseases or malignancies.

METHODS

Preparation of Visceral Adipose Tissue (AT) Mesenchymal Stem Cells (MSCs) for Therapy

MSCs were extracted from visceral adipose tissue. Adipose tissue was obtained as surgical waste during routine ovariectomy of healthy, female, mixed-breed dogs (age: between 7 months and 3 years). Donor dogs underwent all routine vaccinations and were regularly surveyed by veterinarians. Stromal vascular fraction (SVF) and then AT-MSC cultures were generated as previously described (11). The purity and differentiation ability of the resulting MSCs were characterized as described previously (11).

Preparation of AT-MSCs for Therapy

Passage 2 AT-MSCs from two different adipose tissue donors were thawed, cultured for 3 days, mixed, and suspended in 0.5% sodium hyaluronate (TRB Chemedica International S.A. Geneva, Switzerland). Adipose tissue-derived MSCs ($12 \times 10^6 \pm 3.2 \times 10^6$ cells/injection) were transported to the veterinarian clinics in syringes at 4–10°C and injected intra-articularly, within 24 h of dispensing, in a final volume of 1 mL.

Patient Selection and Assessment of the Therapy

This study was approved by the National Scientific Ethical Committee of the National Food Chain Safety Office. All the dog owners signed an informed consent authorizing treatment and were informed of the possible risks of joint injections and potential complications of the procedure.

Fifty-eight dogs suffering from medium or severe osteoarthritis of various joints were included in the study. Severity of OA was evaluated by the participating veterinarians according to a modified method of Black et al. (15) as medium (intermittent but frequent lameness treated with NSAID medication or hyaluronic acid injection) or severe (no cartilage based on the X-ray analysis, continuous pain and lameness, reduction of joint movement, and presence of joint stiffness). The inclusion criteria were recurrent lameness and pain attributed to OA after conventional treatment of dogs (non-steroidal anti-inflammatory drugs, intra-articular injection of hyaluronic acid, arthroscopy, or traditional surgery focusing on debridement and removal of debris from the synovial cavity). Only those dogs were classed as "improved" that did not require further conventional treatment due to their OA during the study period.

The health status, including degree of lameness of the treated dogs was evaluated by the owners using a questionnaire modified from Black et al. (15) (**Supplementary Table 1**), with an occasional participation of the local veterinarian during the first year of follow-up. Further follow-up of up-to 5 years was based on the owners' subjective observations, reporting the degree and frequency of lameness and the possible necessity of pain relief medication since the MSC treatment in personal telephone interviews. The development of other diseases or cause of death was diagnosed by veterinarians during the 5 year follow-up period. The owner-assessed efficacy of the MSC transplantation in OA was evaluated to provide long-term, supportive information on the clinical outcomes following this treatment.

There were no exclusion criteria except for pre-existing, diagnosed cancer.

RESULTS

Overall, fifty-five out of 58 transplanted animals were available for the long-term follow-up. Forty-two animals suffered from elbow OA (**Table 1** and **Supplementary Table 2**); all suffering from elbow dysplasia, except two dogs (aged 12 and 13 years) which had developed cartilage degeneration probably due to their age. Eleven dogs received MSCs into both elbows, while 31 animals obtained transplant into one joint (**Supplementary Table 2**). Thirty-nine dogs were available for the

TABLE 1 | Summary of long-term follow-up after MSC transplantation.

Site of OA	No. of animals included/No. of animals evaluated	Range of age (average) at the time of MSC injection in years	Average age at the time of follow-up (years)	Results at 2.5, 4, or 5-year follow-up		Joint or spinal diseases other than the transplanted joint	Number and cause of death	
				Improved or sporadic lameness and/or medication during weather fronts or extreme activity	No improvement continuous medication for other joint/spinal problems or death		Other than cancer	Cancer
Elbow OA	39*/31**	0.6–10 (3)	7.3	26 (84%)	5	9	8	3
Hip OA	5/4&&	1.5–8 (5.5)	9.7	3	1	2	1	1
Knee OA, dislocation, ligament tear	8/6&&	3–10 (5.6)	10	5 1	1	3	3	-
Hock OA	1	0.8	5	1	-	-	-	-
Ankle OA	2	0.4–1.5 (0.96)	5.5	2	-	-	-	-
Total	55&/44&&	0.4–10 (3.2)	7.5	37 (84%)#	7 (16%)	14	12	4

The condition (lameness, usage of drugs) of the transplanted joint of the same animal was followed up for 2.5, 4, or 5 years depending on the date of transplantation.

 # % = (Improved animal number: number of animals in the study) × 100.

**6 dogs died within 1 year of the survey.*

***3 of them died just before the end of the survey, but their condition was evaluated till death.*

&One dog died within 1 year of the survey and 3 died later but before the end of the follow-up.

&&One dog who died before the end of the survey was evaluated till death.

long-term follow-up, by the end of which the average age of the dogs in the study increased from 3 to 7.3 years.

Sixteen dogs that received the MSC treatment into other joints [knee (8), hip (5), ankle (2), and hock (1)] (one joint in one animal, see **Supplementary Table 2**) were also evaluated in the long-term follow-up (**Table 1**). The average age was 4.8 years at the time of transplantation, increasing to 9 years by the end of the study period.

Thirty-seven dogs out of 44 (84%), were reported by the owners to have reduced lameness and an improved quality of life until the end of the follow-up period or the death of the treated animals. Several dogs improved, and although suffered from other joint problems did not require medication. Seven dogs diagnosed with other joint or spinal problems besides elbow OA and hence receiving continuous medication were also included into the survey period of elbow OA (**Table 1**).

Two of 58 dogs showed a short-term local inflammation after MSC transplantation. This inflammatory reaction resolved within a week and did not affect the long-term improvement in the quality of the dogs' life (data not shown).

Sixteen dogs between the ages of 7 and 13 died during the follow-up period, four of them of cancer (between the ages of 11 and 13) and 12 due to various other diseases (aged 7–12 years) (**Table 1**). Cases of cancer included: melanoma, epithelial cancer, metastatic cancer, spleen-liver cancer (a single case for each tumor-type). Other co-morbidities reported by the owners included: epilepsy, pancreatitis, volvulus, heart failure, ulcers, neurological, and musculo-skeletal problems. None of these syndromes were reported more than twice, suggesting that they may not be associated with the MSC treatment.

DISCUSSION

The aim of this study was to explore whether local intra-articular transplantation of MSC had long-term beneficial effects on lameness and hence improving quality of life of the transplanted animals and whether it caused any serious adverse effects or correlated with an increased prevalence of other diseases.

Our data presented in this paper suggest that MSC transplantation results in improvement of motion. In the current study, 60% of the treated animals belonged to 3 breeds: Golden Retriever (9), Labrador Retriever (16), and German Shepherd (8). This is consistent with these breeds being generally recognized as at high risk of OA (1). The sex of the animals did not correlate with either the coincidence of OA or the death occurring subsequently to MSC injection. Our results presented here are in line with the literature suggesting a beneficial effect of MSC transplantation in OA (11, 16).

In the cohorts included in the long-term survey, most dogs suffered from elbow OA (42 dogs out of 58) of which 40 were diagnosed with elbow dysplasia, hence elbow dysplasia was accepted as the main cause of osteoarthritis in the elbow. Eighty four percentage of the animals maintained an improved condition (no lameness/no medication or sporadic lameness and/or medication during wet weather or with extreme activity) by the subjective assessment of the owners.

Osteoarthritis cases in joints other than the elbow (knee, hip, ankle, and hock) were also evaluated. Eighty four percentage of the evaluated dogs retained their improved condition after the 4-5-year follow-up. It should be noted that the dogs in this mixed group were of higher age than those in the elbow group, and many patients suffered from joint or spinal problems in addition to the treated joint.

MSC transplantation does not appear to be associated with an increase in malignancies or other diseases, and no other adverse effects emerged due to MSC injection. These findings are underpinned by: (1) the published literature that supports that MSC is not a tumorigenic cell type (17, 18); (2) published findings that local injection into the joint results in the adherence of MSCs to the damaged cartilage (19) with no reported evidence for their migration outside of the treated joint. Sixteen dogs out of 58 transplanted animals died during the long-term follow-up: four due to cancer in old age (11–13 years) and 12 from other different causes.

This rate, in spite of the small number of cases overall, is comparable to large-scale disease prevalence statistics in the USA (5.3%) thus does not indicate an association between prevalence of cancer and MSC transplantation (20). Other studies report a prevalence of 3.4–8.63% for melanoma (in dogs the age average was 7, 5 years) in Switzerland and Brazil and 3.4% for internal organ tumors in India over a period of 10 years (21–23). Published literature demonstrates a 4.9% prevalence of death due to heart failure in dogs with a median of 9.9 years in the UK (24), whilst our study reports 3.6%. Of note, the dog population included in our follow-up study is comprised of older dogs, thus, the number of deaths due to tumors is expected to be higher than that in the whole population. In our study, however, the average age of the dogs that died due to tumors was 12.2 years, while in a previous report from the USA involving golden retrievers it was 9.83 years (25).

The potential use of allogeneic MSCs for the treatment of various diseases has also been piloted, including humans and horses, for instance, for indications such as cardiac damage post-myocardial infarction and wound healing (26, 27). However, whilst long-term safety data is still very sparse, evidence is now emerging on a convincing safety profile for the use of allogeneic MSCs in cardiac regeneration (28) and autoimmune disease in man (29) as well as in osteoarthritis in horses (30). Our current report is in alignment with this broader literature and further highlights the potential for the use of allogeneic MSCs in a variety of diseases.

In summary, our data suggest that intra-articular injection of allogeneic MSCs may provide a long-term improvement in lameness secondary to OA, based on subjective reporting of the owners, without the risk of long-term adverse effects on health. Thus, we conclude that MSC treatment could be an effective and safe long-term therapy for canine OA.

Though the results overall suggest that MSC treatment is beneficial for dogs suffering from osteoarthritis, evaluation is limited by the fact that the improvement was subjectively compared to the initial state by owners. To overcome these limitations, a follow-up with rigorous orthopedic examination performed by veterinarians will be recommended.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by Csongrád County Committee on the Food Chain and Animal Health, Government Office of Csongrád County, Rákóczi tér 1, Szeged 6722 Hungary. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

EK-P: preparing MSCs for therapy. LH: organization of contacts with vets and owners. PC and EK-T: Evaluating results. VS: keeping contact and interviewing the owners. EM: leading the study. All authors: contributed to writing the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.00510/full#supplementary-material

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Conflict of Interest: EK-P was employed by company Stem CellX Europe Limited. PC and EK-T are shareholders of Stem CellX Europe Limited and PC is a shareholder of Assentra Limited.

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BRIEF RESEARCH REPORT
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Comparison of Two Distraction Devices for Assessment of Passive Hip Laxity in Dogs

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Canine hip dysplasia is the most common orthopedic developmental condition in the dog and early hip laxity is the main risk factor. The importance of hip laxity in young animals in the development of hip dysplasia is unanimously recognized among researchers and veterinarians due to its medical applicability in terms of disease control and prevention. In the market, there is some certified hip distractors to promote joint laxity. However, the clinical use of some of these distractors complies with a set of usage rules, that can limit its medical application. In this study was compared the technical quality of radiographs and hip distraction using a certified hip distractor (CertD) and Dis-UTAD in 104 dogs (208 joints). The mean pelvic tilting of 1.5 ± 1.6° and 1.5 ± 1.8° were similar when using the CertD and the Dis-UTAD distractors, respectively ($P > 0.05$). In the CertD sample, the mean hip distraction index (DI) was 0.46 ± 0.17 and in the Dis-UTAD 0.46 ± 0.16; the mean DI differences was 0.001 ± 0.045, resulting in a non-significant paired *t*-test ($P = 0.65$) and a significant intraclass correlation coefficient of 0.96, with the 95% lower limit confidence interval of 0.95 ($P < 0.05$). The statistical power analysis showed a very low distraction index difference effect size. The results suggest that the statistical reproducibility of CertD hip distraction by the Dis-UTAD and the DI mean differences of 0.001 might be considered without clinical importance. The Dis-UTAD might be considered adequate to promote dog hip laxity.

Keywords: hip laxity, distraction index, reproducibility, PennHip, Dis-UTAD, dog

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INTRODUCTION

Canine hip dysplasia (CHD) is a complex, inherited, polygenic trait disease influenced by multiple environmental factors, which was first identified in dogs by Schnelle in 1935 (1–3). CHD is considered as one of the most common orthopedic developmental conditions in dogs that lead to a debilitating secondary hip osteoarthritis (4). Although the etiology of CHD is not completely understood, increased laxity of the hip joint is the most frequent early cause reported and usually results in secondary osteoarthritis (OA) (5). CHD is a challenging disease to prevent, diagnose, and manage. Clinical signs such as decreased activity, difficulty in rising, "bunny hopping" gait, hind limb lameness, and hip pain support the suspicion of the disease (6). The actual diagnosis is confirmed radiographically if characteristic signs are evident on standard hip extended view (HEV)

in dogs over 1 year of age (4). There is not an adequate molecular diagnosis for hip dysplasia (3); therefore, radiographic diagnosis has been essential for the selection of breeding stock and is based on two main key features: the detection of signs of degenerative joint disease or the diagnosis of early hip joint laxity (HJL) (7, 8). Although HEV has been shown to be a valuable tool in evaluating the presence of OA, it can severely underestimate HJL because of the non-physiological tensioning of the pelvic muscles and twisting of the joint capsule (5). Distraction–stress radiography techniques are used to better estimate the degree of passive HJL through the calculation of distraction index (DI) (5, 9). In the hip distraction view, the femoral heads are displaced laterally by the use of a custom-designed device (distractor) placed between the legs that acts as a fulcrum on the femur at the level of the ventral aspect of the pelvis (5). The DI is obtained by dividing the lateral femoral head displacement by its radius; a DI of 0 represents absolute joint congruity and a DI of 1 represents complete joint luxation (5). The hip joint distractors PennHIP (5, 7) and "FSA—Fondazione Salute Animale" (9) have been used in published works to obtain hip distraction views.

The purpose of the present study was to compare the technical quality of radiographs and hip distraction using the CertD and the Dis-UTAD, a hip distractor developed at the University of Trás os Montes e Alto Douro (UTAD).

MATERIALS AND METHODS

Animals

In this prospective study, 104 dogs (58 females and 46 males) from five different breeds (68 Estrela Mountain dogs, 12 Transmontano Cattle Dog, 12 Portuguese Pointer Dog, 11 Rafeiro do Alentejo, and one Barbado da Terceira) were presented at the Veterinary Teaching Hospitals of University Lusófona de Humanidades e Tecnologias and University of Trás-os-Montes and Alto Douro between the years of 2014 and 2019 and screened for passive hip laxity. The recorded data included breed, age at the time of radiography, sex, and body weight. The inclusion criteria were that dogs had to be from Portuguese breeds, between 4 and 12 months of age at the time of the exam, and presenting normal musculoskeletal development upon clinical examination. The minimum sample size was estimated using a *t*-test table and selecting a statistical significance of 0.05, a medium variable effect size of 0.4, and a statistical power of 0.8, which indicated a sample of 99 observations (10).

All the radiographic examinations were performed with the dog owner's consent and all the animal procedures were undertaken as part of the work described in this study, performed in compliance with the regulations of our institutions (no. 1044-e-DCV-2018) and in accordance with the Portuguese and European regulations for animal use and care (European Directive 2010/63/EU and National Decree–Law 113/2013).

Radiographic Procedure

The radiographs were performed with the dogs under deep sedation using medetomidine (Domitor: Orion Corporation, Espoo, Finland) and butorphanol (Torbugesic Injectable: Fort Dodge Veterinaria, Girona, Spain) administered intravenously. The sedation was reversed with atipamezole hydrochloride (Antisedan: Orion Corporation, Espoo, Finland) intramuscularly. Radiographs were obtained in the same sequence with the dogs in dorsal recumbency on the X-ray table: first the HEV and then two distraction views with the distractor device placed between the hind limbs to promote passive hip laxity, first using the CertD and followed by the second distraction view using the Dis-UTAD. The Dis-UTAD is a modified Vezzoni distension device with an isosceles trapezoid shape (9); it has an external rubber component and a polyethylene plate in the interior that gives it longitudinal flexibility and transverse stiffness (11). With the dog in dorsal recumbency, hip distraction was achieved in a similar way to the PennHIP and Vezzoni techniques (5, 9). Both femurs were adducted by the examiner symmetrically in a neutral position (+/−10 degrees) against the distractor, fixed on the animal with the support of two cylindrical sandbags weighing ∼4 kg each, one at the front and one at the distractor's back (**Figure 1**). On the distraction radiograph, the pelvis and the distractor should appear centered and symmetrical, and the more pronounced lateral band opacity of the distractor overlaps the femoral heads (**Figure 2**). The hip distractor fixation with sandbags was already described previously to avoid the exposure of the examiner's assistant to ionizing radiation (12). The radiographs were taken by veterinarians with experience in hip stress views (AS and MG).

Positioning Evaluation and Hip Laxity Measurement

Technical radiographic positioning analysis was performed by estimating the grade of pelvis tilting (y) and measuring the asymmetry of the obturator foramen width (OFW) (x), $y = 1.6x - 0.9$ (13). For the hip laxity measurements, a dedicated semiautomatic software was used as previously described (14). The DI was calculated by dividing the distance between the centers of the femoral head and the acetabulum by the radius of the femoral head, as described previously (5). Both measurements of pelvic tilting and DI were performed in two independent sessions by JM and AS, respectively.

Statistical Analysis

Statistical analysis was performed using the computer software SPSS (SPSS Statistics for Windows Version 23.0: IBM Corp., Armonk, NY, USA). The data analysis was performed on joints individually. The paired *t*-test and the intraclass correlation coefficient (ICC) were used in comparing the pelvic tilting and the DI of both hip distractors in order to evaluate Dis-UTAD's reproducibility (15, 16). A value of $p < 0.05$ was considered to be statistically significant. The null hypothesis was that the mean difference between paired observations was zero (10). The size effect and the statistical power were estimated to evaluate the ability of our sample to detect variable differences on each distractor set (10).

RESULTS

The dogs' age ranged from 4 to 11 months (mean ± standard deviation, 6.0 ± 2.1 months), and body weight ranged from 13.5 to 54 kg (mean, 24.7 ± 8.6 kg). In the CertD sample,

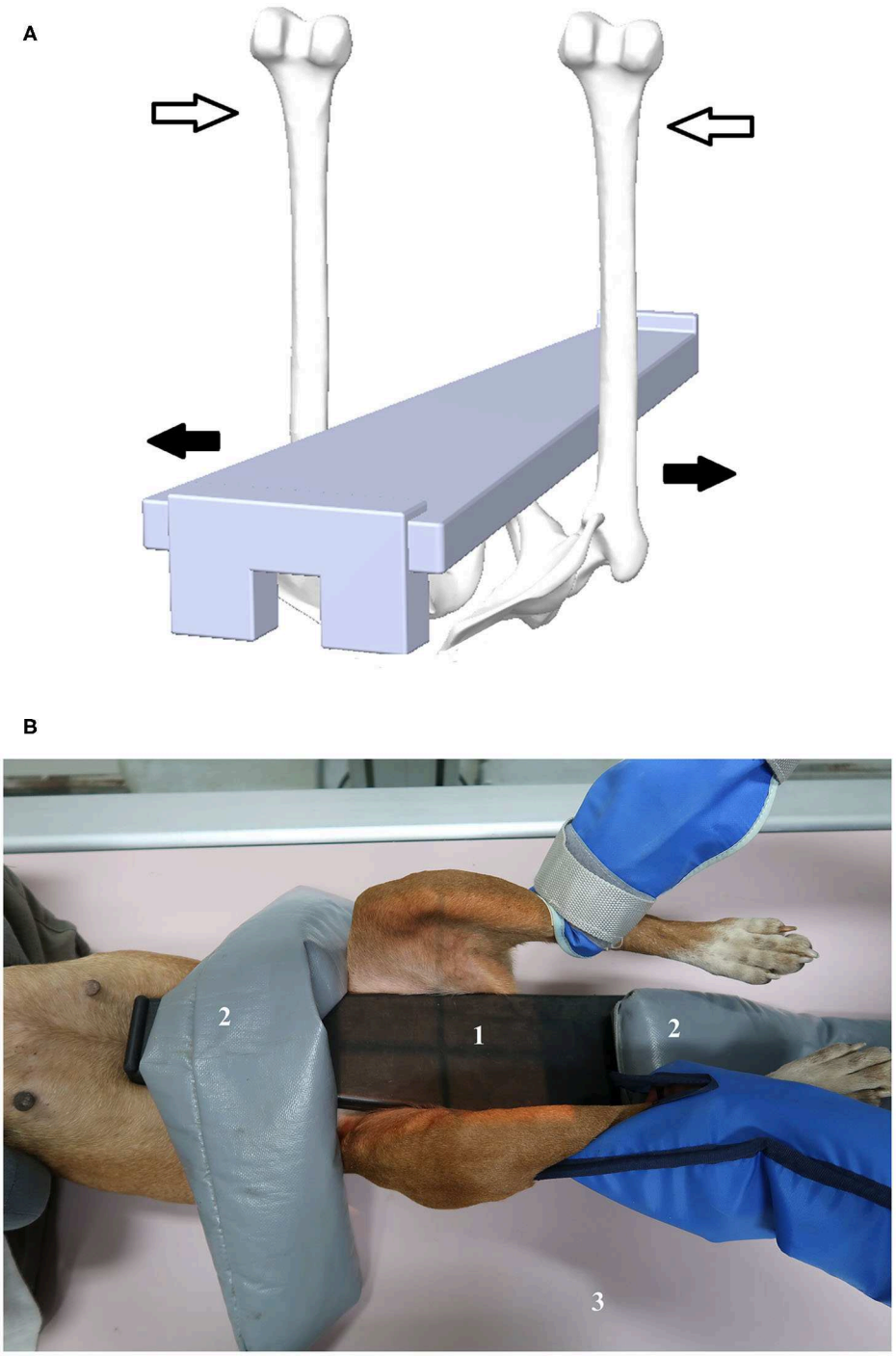

FIGURE 1 | The Dis-UTAD is a modified Vezzoni distension device. **(A)** Illustration outlining the rear view of the dog and the Dis-UTAD. The open arrows represent the medial force exerted by the examiner, pushing the femurs against the distractor, and the full arrows represent the resulting hip distraction force. **(B)** Dis-UTAD positioned on the animal, fixed by the support of two sandbags. 1, Dis-UTAD; 2, sandbags; 3, X-ray table.

the pelvic tilting ranged from 0 to 6.5° (mean, 1.5 ± 1.6°) and the DI ranged from 0.16 to 0.88 (mean, 0.46 ± 0.17). In radiographs obtained with the Dis-UTAD, pelvic tilting ranged from 0 to 6.3° (mean, 1.5 ± 1.8°) and the DI ranged from 0.12 to 0.88 (mean 0.46 ± 0.16). Comparing CertD and Dis-UTAD, for pelvic tilting the mean of the difference was 0.04 ± 1.9° and for DI it was 0.001 ± 0.045°, and the paired *t*-test was not statistically significant in both evaluations, being $P = 0.84$

and 0.65, respectively (**Table 1** and **Figure 3**). The ICC between both DI samples for single measures was 0.96 (95% confidence interval, 0.95–0.97).

DISCUSSION

Hip joint laxity is the main risk factor for the development of degenerative joint disease in dogs and is associated with high heritability (3, 8). Therefore, the use of DI breeding selection is highly recommended in the control programs of CHD (4). The clinical achievement of the distraction view requires some specific training and PennHIP has free online courses available. However, the clinical use of some hip distractors complies with a set of imposed rules that can limit its clinical usage (9, 17). The Dis-UTAD was developed to overcome some restrictions in hip distractor availability and intended, for interested veterinarians, as an adequate alternative in the assessment of dog's hip joint laxity. However, like the usage of other hip distractors, a previous practical training is recommended to perform adequate hip distraction views as well as for the distraction index measurement (14).

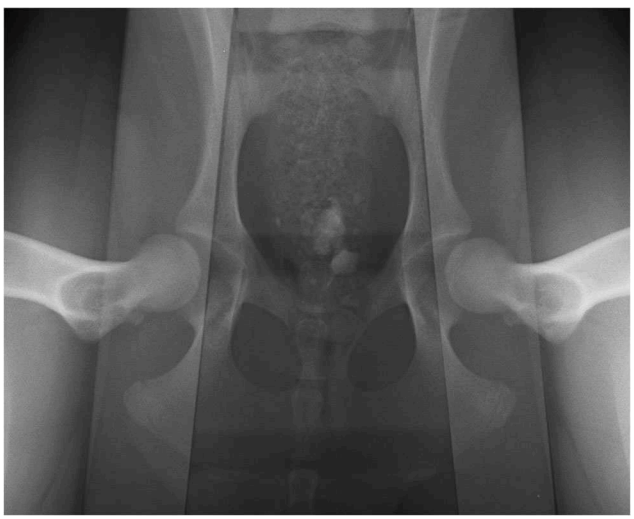

FIGURE 2 | Hip distraction view using the Dis-UTAD distractor. The pelvis and the distractor are centered and symmetrical, and the more pronounced lateral band opacity of the distractor overlaps the femoral heads.

In this work, the OFW was used to evaluate the grade of pelvis tilting and not the iliac horizontal diameter as recommended in a previous work that used the HEV (13) because, in some radiographs of our sample, the X-ray collimation did not allow the observation of all sacroiliac joints. Nonetheless, this study showed also a good correlation between OFW and pelvic tilting (13). Our work shows that the degree of pelvic tilting using Dis-UTAD is similar to the one that we obtain by using the CertD. The mean degree of pelvic tilting in our samples (1.5°) was slightly higher than those in other works where conventional hip ventrodorsal view was used (13, 18). There are no previous published distraction hip studies where the degree of pelvic rotation has been evaluated. Theoretically, the tilting of the pelvis should not have much interference in DI measurement since this variable results from two spherical anatomical structures in a similar dorsal dog anatomical plane and relatively close to the center of the X-ray beam. Radiographic spatial distortion is especially important in the periphery of the X-ray beam and when the reference structures are at different distances from the radiographic film (19).

The non-significant *t*-test and the ICC of 0.96 with a lower limit of the 95% CI $\geq$ 0.75 indicate that there is no bias, a strong association between the hip distraction promoted by both distractors and statistical reproducibility and interchangeability (16). However, the low statistical test power does not allow us to reject the false null hypothesis (10). As the mean DI difference effect size is very low (0.07), we will need a sample of more than 1,500 animals to obtain enough statistical power (0.80) to demonstrate that the DI differences are not due to the Dis-UTAD distractor (10). However, when the mean variable differences are exceedingly small, they can be considered without medical importance (20, 21). There is no statistical test powerful enough to detect variable differences between samples with a mean of 0 since an infinite sample would be needed for comparison (21). The DI measurement differences included also examiner and scoring errors (22), which are difficult to differentiate. A similar reproducibility of the PennHIP method results was obtained in a recent study using the Vezzoni Modified Badertscher distractor (9). The longitudinal flexibility of the Dis-UTAD allows good adaptation to the dog's body and the sandbags stabilize it adequately, resisting better to the examiner's medial force on the hind limbs. Distractor stabilization is also needed in the PennHIP distractor using sandbags (12) or the help of an assistant (5), which we do not recommend because it increases human exposure to ionizing radiation.

TABLE 1 | Paired variable differences between CertD and Dis-UTAD.

Variable	*N*	Paired differences						*r*	Effect size	Power
		Mean	SD	SEM	95% CI		*P*			
					Lower	Upper				
PT (°)	104	−0.04	1.87	0.18	−0.4	0.33	>0.05	0.4	0.02	0.05
DI	208	0.001	0.045	0.003	−0.005	0.008	>0.05	0.96	0.03	0.07

°, degrees; CI, confidence interval; DI, distraction index; N, number; P, statistical significance; PT, pelvic tilting; r, Pearson correlation; SEM, standard error of the mean; SD, standard deviation.

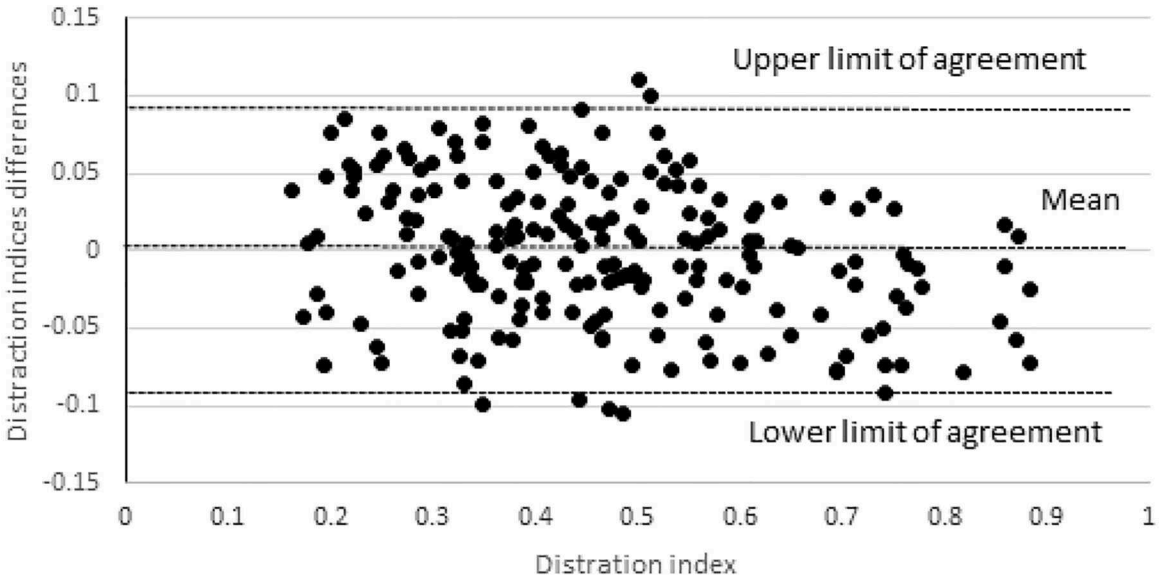

FIGURE 3 | Differences between the distraction index obtained in distraction radiographs using the certified distractor and the Dis-UTAD. The horizontal lines represent the mean of the differences (0.001) and the upper and the lower 95% limits of agreement, ∼0.09 and -0.09, respectively.

The higher lateral band opacity of the distractor, overlying the femoral heads, gives the examiner a good idea of the symmetry of positioning and distraction level (**Figure 2**). It is recommended to repeat the examination when this does not happen. The rear Dis-UTAD thicker component with a central hole allows table support, accommodates the tail of the dog, and allows a more horizontal use of the distractor, characteristics that facilitate the hip distraction process. The heterogeneity of our sample (animals of about 10–50 kg) also shows that Dis-UTAD has the ability to promote adequate hip distraction in small and large dogs using its cranial or caudal part in distraction, respectively. Nonetheless, the small number of breeds can be pointed out as a limitation of this study. The DI ICC in this study (0.96) was higher than the within- (0.94) and between-examiner (0.91) DI repeatability of previous studies using the PennHIP distractor (17) and similar to other studies that evaluate the reproducibility of PennHIP DI measurements (22). These facts may be associated with the examiner's expertise and the reliability of the DI measurement method or may be related to the size or the type of the sample used. The dedicated semiautomatic software used in hip laxity measurements was already used in a previous work, which proved to be effective (14).

This and other recent studies show that joint laxity can be reliably quantified with the use of different distractors and methods (9), and there are scientific and technical conditions for extending their use in breeding selection and for preventive CHD management purposes. The recognition of the importance of hip laxity in young animals in the development of hip dysplasia is unanimous among researchers and veterinarians (5, 7, 9, 23, 24), so its medical applicability in terms of disease control and prevention should be promoted between veterinarians, owners, and dog breeders. However, it should be kept in mind that the success of hip dysplasia control programs depends more on the knowledge of the breeding population than on the dog alone, and databases with reliable medical information are essential (25, 26).

CONCLUSIONS

Pelvic tilting and DI measured using the Dis-UTAD showed the statistical reproducibility of the CertD measurements. The mean DI difference of 0.001 might be considered without clinical importance. The Dis-UTAD might be considered adequate to promote dog hip laxity.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by Comissão de Ética da Universidade de Trás-os-Montes e Alto Douro (CE-UTAD). Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

AS contributed to the acquisition of data and drafting of the manuscript. SA-P contributed to the critical revision of the manuscript. JM contributed to the acquisition of data and critical revision of the manuscript. BC contributed to the concept/design and critical revision of manuscript. MG contributed to the

concept/design, acquisition of data, data analysis/interpretation, and drafting of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2020.00491/full#supplementary-material

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ORIGINAL RESEARCH
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Measurement of the Femoral Anteversion Angle in Medium and Large Dog Breeds Using Computed Tomography

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To promote the development of an optimally functional total hip prosthesis for medium and large dog breeds, accurate measurements of the normal anatomy of the proximal femur and acetabular retroversion are essential. The aim of the current study was to obtain precise normal values of the femoral anteversion angle using computed tomography on cadavers of mature dogs with normal hip joints of both medium and large breeds. Based on the length of their femora 58 dogs were allocated either to group I: ≤195 mm or group II: >195 mm. In the study the femoral anteversion angle (FAA) was measured on each femur using multi-slice spiral computed tomography (CT). The data were processed as multi-planar and three-dimensional reconstructions using Advantage Workstation software. The CT measurements showed that the mean ± standard deviation (SD) FAA of group I was 31.34 ± 5.47° and in group II it was 31.02 ± 4.95°. There were no significant mean difference associations between the length of the femur and the femoral neck angle in either group ($P > 0.05$). The data suggest that a prosthesis FAA of 31 degrees would be suitable for a wide range of dog sizes.

Keywords: computed tomography, total hip replacement, canine, femoral anteversion angle, femoral morphology

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INTRODUCTION

The hind limb is frequently affected by several orthopedic diseases, such as hip dysplasia especially in medium and large dog breeds (1–6). The femoral anteversion angle (FAA) is a significant and frequently used measure for understanding the orientation of the proximal end of the femur (7–9). It plays an important role in the assessment of the health of the hip joint due to its involvement in the development of coxarthrosis in dogs (1, 10). The FAA is defined as the angle formed by the intersection of the axis of the femoral neck and the transcondylar axis of the femur, which is the axis parallel to the medial and lateral posterior edges of the condyles in the condylar plane (4). It indicates the degree of torsion of the femoral neck and head cranially and represents external rotation of the femoral neck and head relative to the distal femur (11–13). It is important biomechanically in the transfer of forces from the femur to the acetabulum (14). In a larger than normal FAA, the lever arm between the center of the femoral head and the greater

trochanter is shortened (14). Thereby, the pressure, that acts on the femoral head in the acetabulum, is higher. Anatomists and surgeons have long been interested in the FAA since it is considered an important factor for hip joint stability (1, 2, 4, 8, 9, 15).

Surgical treatment of serious hip joint problems often requires total hip arthroplasty. Both the femoral neck angle and the femoral anteversion angle, that describe the relationship between the femoral head, neck and the femur shaft, must be taken into account in the development of hip endoprostheses in order to reduce the risk of hip luxation following the implantation of the prosthesis (16). Using a total hip replacement prothesis with an inappropriate FAA value may result in premature wear and loosening between the prothesis stem and the internal surface of the femoral shaft due to the increased pressure which finally cause failure of the prosthesis.

Many different methods have been used to determine the FAA, including standard radiography (1, 12, 17), biplanar planar radiography (4, 7, 18–21), computed tomography (CT) (9, 12, 22, 23), magnetic resonance imaging (24), three-dimensional modeling (25) and three-dimensional (3D) laser scanner techniques (8, 26).

Using single standard radiographic imagery to measure the FAA does not truly reflect spatial relationships between pertinent landmarks, due to a lack of depth information (27). CT imaging is considered to be a reliable and an accurate method for measuring the FAA because it allows accurate 3D volumetric femoral reconstructions of the femur and avoids artifacts due to incorrect positioning thus improving the precision of FAA measurements (20, 28–30) with average errors of 0.45° (30).

The main aim of this work was to use CT to obtain precise data of the femoral anteversion angle in cadavers of medium and large dog breeds in support of the development of an optimally functioning total hip replacement prothesis. In addition we provide a detailed description of the methodology using CT to measure the femoral anteversion angle.

MATERIALS AND METHODS

The cadavers used in this study have been reported earlier in a previous article where the femoral neck inclination angle was studied (31).

Femora from 58 cadavers of orthopedically healthy adult dogs of medium and large breed size were studied using computed tomography. The dogs used in this study were obtained from the Small Animal Clinic of the Free University of Berlin. The dogs had either died or were euthanased for reasons unrelated to this study. For each individual dog the research ethics code of the institution was met and accompanied by written consent from the dog's owner.

Post-mortem examination was conducted on each dog to establish the absence of orthopedic abnormalities and disease. The Ortolani and Barlow tests were conducted immediately post-mortem. Radiography and CT examination of the hip joint was conducted post-mortem immediately after the death to establish the absence of hip joint dysplasia. The dogs used in this study had no clinical history of pelvic limb lameness. Dogs with orthopedic abnormalities or signs of hip joint disease were excluded from the study.

The dogs used in this study were assigned into two groups according to the length of their femora measured in CT (32).

The CT scanning was conducted at the Small Animal Clinic, Düppell, Free University of Berlin. The CT scanning of the femora was done at a setting of 0.3 mm slice thickness, multi-slice spiral "Lightspeeds" QXi (General Electric Healthcare, GE), 120 kV, 130 mAs. The dogs were positioned in dorsal recumbency on the CT scanner table. The pelvic limbs were pulled back and tied at the tarsal level with adhesive straps (Tesa AG Humburg) to ensure that the femora were parallel to each other and parallel to the CT scanner table. Advantage Workstation software (Advantage Workstation 4.2, GE Healthcare) was used to analyse the images. The data record was processed as multi-planar and three-dimensional reconstructions using Advantage Workstation software.

The sequence of measurements were done in the following order as some measurements were reliant on values of earlier measurements: determination of the axis of the femoral shaft, length of the femur, center of the femoral head, axis of the femoral neck, condylar axis, femoral anteversion angle. All measurements were performed by an experienced veterinarian and repeated after 24 h. The mean of the two measurements of the FAA was used to ensure data accuracy.

Medullary Axis and Length of the Femur

To ensure consistency in femoral measurements, an exact sagittal plane view was obtained by aligning the caudal aspects of both femoral condyles, and to avoid cranial or caudal inclination of the femur, the femoral axis was identified as the line connecting the three central points shown in **Figure 1a** and was placed vertically (**Figure 1a**). From here the femur was rotated exactly 90° cranially to be able to obtain an accurate frontal plane view of the femur without external or internal rotation (**Figure 1b**).

In the sagittal and frontal planes, the center of the intracortical width was created at the narrowest point of the femoral shaft. Using similar methodology, additional central points were placed 2 cm proximal and 2 cm distal. The axis of the femoral shaft was identified as the line connecting the three central points (**Figures 1a,b**). Using a three dimensional model in a frontal view, the length of the femur was determined to be the line parallel to the femoral axis that connects the orthogonal lines at the most proximal point of the femoral head and at the most distal end of the femoral condyles (**Figure 1c**).

Center of the Femoral Head

Using a 3D transverse plane, the center of the femoral head was identified by using annotation software to generate concentric circles of best fit and superimpose these onto the femoral head (**Figure 2a**).

Axis of the Femoral Neck and the Condylar Axis

In the transverse femoral neck planes the lesser trochanter appears at the transition from the medial to the caudal contour

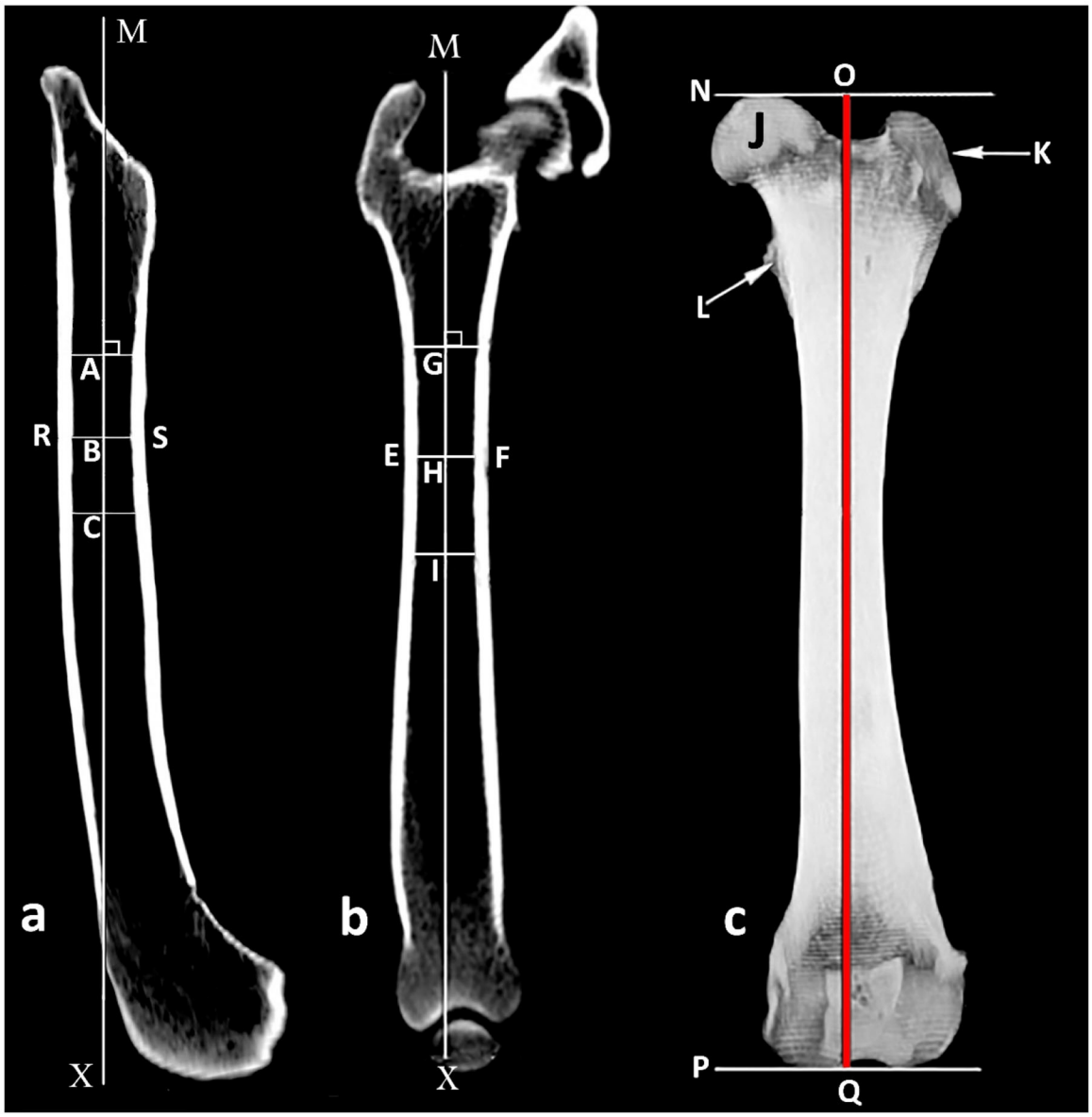

FIGURE 1 | Determination of the medullary axis of the femoral shaft. **(a)** Sagittal plane view of the femur where line RS represents the intracortical width at the narrowest point of the femoral shaft and B is its central point; A and C are central points 2 cm proximal and 2 cm distal to (B) respectively; MX is the medullary sagittal axis. **(b)** Frontal plane view of the femur where EF represents the intracortical width at the narrowest point of the femoral shaft and H is its central point; G and I are central points 2 cm proximal and 2 cm distal to H, respectively; MX is the medullary frontal axis. **(c)** Frontal view of the femur where N and P are the proximal and distal orthogonal lines to medullary axis, respectively; J femoral head; K great trochanter; L lesser trochanter; OQ length of the femur.

of the femur and disappears in more distal sections. A computer-generated circle was placed in the section with the maximum extent of the lesser trochanter and the center of the circle was determined and presents the base of the femoral neck (**Figure 2b**). The axis of the femoral neck was defined as the line passing from the center of the femoral head to the base of the femoral neck in the transverse view of the femur and remained visible on the monitor (**Figures 2a**, **3a**). More distally, the sectional view with the maximum caudal curvature of the condyles was defined to represent the condylar axis (**Figure 3a**). The FAA was measured between the femoral neck axis and the condylar axis (**Figure 3b**).

Statistical Analysis

The statistical analysis was based in the comparison of FAA in the two groups with different femoral length. The intra-class correlation coefficient (ICC) and Kendall's tau was used in order to evaluate the intra-observer independent measurement repeatability. The Pearson correlation was used to study the association between the length of the femur and the

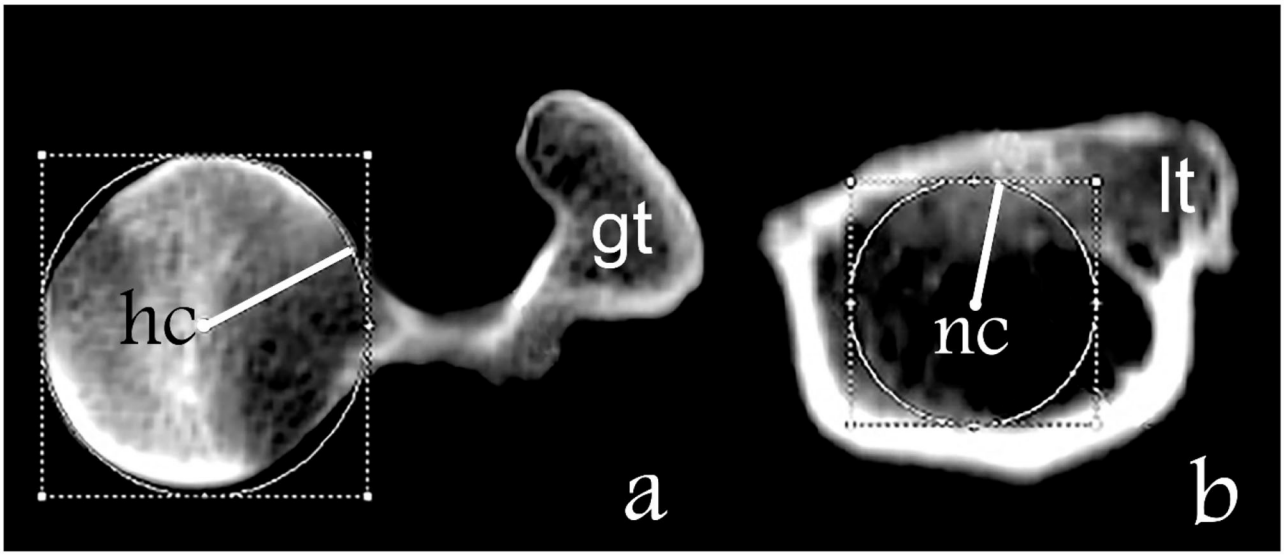

FIGURE 2 | Determination of the axis of the femoral neck. **(a)** Transverse plane of the proximal femur where "gt" is the great trochanter and "hc" is the center of the femoral head. **(b)** Transverse plane of the proximal femur on the level of the lesser trochanter where "lt" is the lesser trochanter and "nc" is the center of the base of the femur neck.

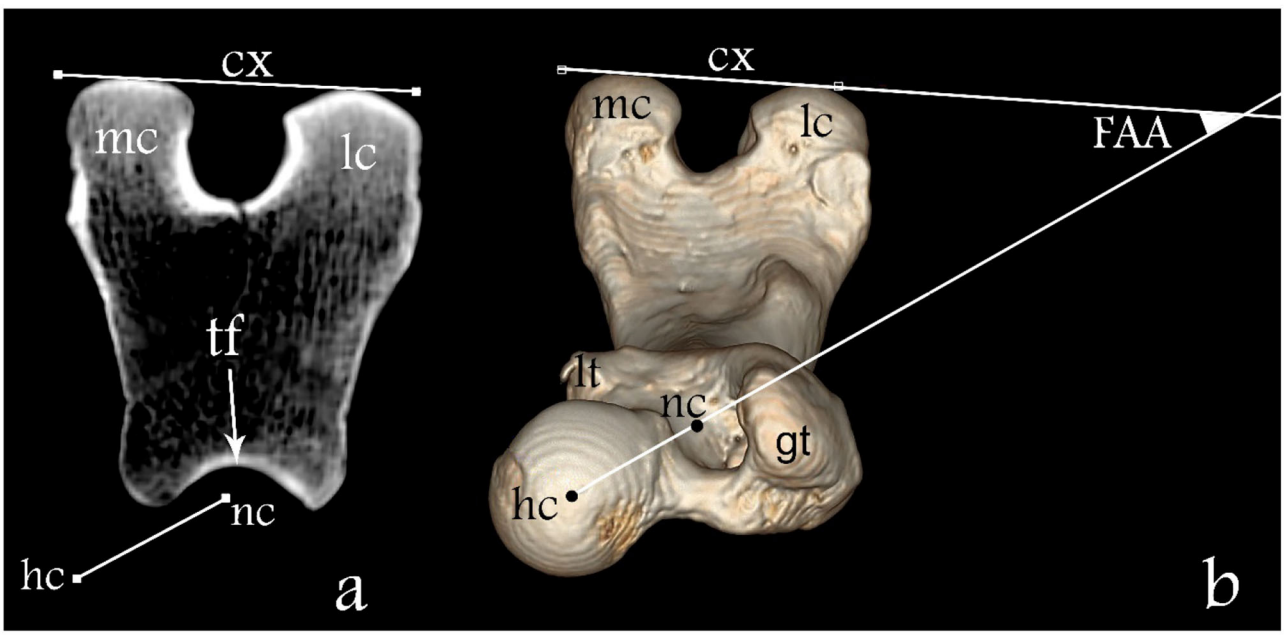

FIGURE 3 | Determination of the FAA. **(a)** Transverse plane overlap view of the distal femur and **(b)** three-dimensional dorsoventral view of the femur. Here "hc" represents the femoral head center, "nc" the femoral neck center, "tf" the femoral trochlea, "mc" medial condyle, "lc" lateral condyle, "gt" greater trochanter, "lt" lesser trochanter; line between "nc" and "hc" femoral neck axis and line "cx" condylar axis. The FAA angle is defined between lines "nc-hc" and cx.

femoral anteversion angle. Pearson and Kendall's tau results of −1 or 1 indicate perfect negative or positive association between variables. A *P*-value smaller than 0.05 was considered significant. The statistical analysis was performed using the Statistical Packages for Social Science software (SPSS Inc. Version 26, Chicago II, USA). Values were reported as mean +/– standard deviation.

RESULTS

In this study a total of 116 femora were measured from 58 medium to large breed dogs. Twenty-three dogs were excluded from the study due to orthopedic abnormalities or signs of hip joint disease which had been detected. The most common breed measured was the German Shepherd followed

FIGURE 4 | Boxplot with medians and data ranges representing the Femoral Anteversion Angle (FAA) in relation to the Length of the Femur for Group I and Group II.

by Staffordshire Terriers, Boxers, Rottweilers, Bullmastiffs, and Weimaraners.

Dogs were divided into two groups according to the length of their femora. Group I ($n = 25$) included all the dogs with a femoral length ≤195 mm. Group II ($n = 33$) included all the dogs with a femoral length >195 mm.

The age of dogs in Group I ranged from 2 to 16 years old, mean 7.6 ± 4.15 years and in Group II ranged from 1.5 to 16 years old, mean 8.4 ± 3.95 years. The body mass of dogs in Group I ranged from 17 to 45 kg, mean 27.8 ± 7.53 kg, and in Group II ranged from 22 to 60 kg, mean 42.3 ± 8.37 kg.

All the measurements performed in the two independent sessions had adequate repeatability as the Kendall's tau test showed strong correlation (tau = 0.956, $P = 0.000$). The femur length in Group I was 175.29 ± 12.29 mm and in Group II 213.44 ± 15.77 mm.

The mean values of FAA obtained in this study were 30.99 ± 4.02° for Group I and 31.58 ± 5.09° in Group II. No correlation was found between the length of the femur and the FAA ($P = 0.136$) (**Figure 4**).

DISCUSSION

Because the dogs used in this study varied in their nutritional status and history, body mass was not included as a morphological parameter (32). The medium to large breed dogs used in the present study were assigned to two groups based solely on the total length of their femur (32). The FAA measured in this study shows no significant difference between group I and group II. Likewise, there was no correlation between the length of the femur and the FAA (**Figure 4**). This is consistent with the results of Palierne et al. (32).

In adult medium and large dog breeds with normal hip joint morphology, the FAA has been measured using several different imaging methods as well as anatomical preparation and reported in the literature to be within the range of 7.6 to 34.2° (3, 4, 7–9, 12, 23, 24, 33–35). The results reported vary greatly in these studies (**Table 1**). However, there are many relatively common congenital and developmental conditions where the FAA deviates significantly from the normal such as canine hip dysplasia associated with a larger than normal FAA, that tends to rotate the femoral head out of the acetabulum (1, 2). The different measurement methodologies as well as the body size, age profile, gender, and breeds of dog populations, may explain the different results (15, 27).

Accurate measurement of the FAA using classical radiography relies on precise positioning of the femur to obtain a true axial projection of the femur from distal to proximal, which is technically challenging due to the difficulties encountered in patient positioning (12). Often multiple attempts are necessary; consequently such radiographic studies can often be time-consuming (12).

Due to the complex three-dimensional configuration of the femur, CT imaging is considered to be the most reliable and accurate method to measure the FAA (9, 12, 20, 26, 28, 29). This allows accurate 3D volumetric femoral reconstructions of

TABLE 1 | Mean (SD) femoral anteversion angle reported in dogs by other studies, measured by standard radiograph (RAD), computed tomography (CT), magnetic resonance imaging (MRI) and anatomical preparation (AP).

Authors	N	Method	FAA (SD)
Adams et al. (8)	five mongrel dogs	3D scanner and 3D animation software	23.4° ± 3.5
Bardet et al. (19)	15 mixed, medium to large	Fluoroscopic method	31.31°
Bardet et al. (19)	15 mixed, medium to large	RAD biplanar	30.8°
Bloebaum et al. (34)	21 greyhound	RAD biplanar	27° ± 6.3
Dudley et al. (12)	nine medium to large	RAD, Fluoroscopic method	16° ± 6.4
Dudley et al. (12)	nine medium to large	CT	19.6° ± 7.9
Dudley et al. (12)	nine medium to large	AP	18.9° ± 5.4
Ginja et al. (23)	23 estrela Mountain Dogs, 7–8 week	RAD biplanar	29.9° ± 4.8
Ginja et al. (23)	23 estrela Mountain Dogs, 7–8 week	CT	30.4° ± 4.2
Griffon et al. (7)	160 labrador Retrievers	RAD biplanar	29.67° ± 6.44
Hauptman et al. (3)	75 medium to large	RAD biplanar	15.2°
Kaiser et al. (24)	40 small, medium to large	MRI	7.6° ± 5.5
Kara et al. (9)	75 mixed breeds	CT	26.86° ± 11.46
Löer (36)	large breeds	CT	33.8°
Löer (36)	small breeds	CT	33.2°
Madsen and Svalastoga (37)	41 medium to large	RAD biplanar	30°-43°
Mahringer, (38)	105 medium to large	AP	33° ± 8.66
Montavon et al. (4)	30 mongrel dogs, medium to large	RAD biplanar	31.3° ± 6.2
Martins et al. (21)	126 young normal joints	RAD biplanar	31.4° ± 4.8
	106 young abnormal joints		32.6° ± 4.9
	158 adult normal joints		26.4° ± 4.5
	232 adult abnormal joints		27.7° ± 5.0
Nunamaker et al. (1)	34 various breeds adults	RAD, Fluoroscopic method	26.97° ± 6.52
Palierne et al. (35)	82 medium to large	RAD biplanar	30° ± 6.32
Palierne et al. (32)	206 small, medium to large	RAD biplanar	29.40° ± 6.35
Savio et al. (26)	16 medium to large	3D scanner and design software	45° ± 4.5
Schawalder et al. (11)	50 medium to large	RAD biplanar	30.1°
Sumner et al. (33)	15 medium to large	RAD biplanar	34.2° ± 5.7

the femur and obviates artifacts related to animal position and thereby increases the precision of the FAA measurement (12, 20, 28, 29) and can be used for clinical or research purposes without the need of additional radiographic exposures (12, 23).

The patient preparation and the time required for radiographic and CT examinations are similar (23). Even using the same imaging technique could result in different values, due to the different methodologies used to estimate the center of the base of the femoral neck (27). Minor variations in radiographic positioning and selection of landmarks affect the correctness and variability of radiographic measurements (13).

In the present study, the precise FAA was obtained using a CT scan data set of 116 femora of 58 mature dogs, all free of hip dysplasia. Multi-slice spiral computed tomography and Advantage Workstation software were used for the analysis. A set of five landmarks; the center of the femoral head, center of the base of the femoral neck, lesser trochanter, medial and lateral aspect of the femoral condyles were found to be readily identifiable and suitable for our CT measurements.

In this study the mean value of the FAA in dogs with a femoral length of between 145 and 195 mm (group I) is 30.99 ± 4.02° and in dogs with a femoral length of between 196 and 240 mm (group II) is 31.58 ± 5.09°. The mean FAA reported in the present study are in close agreement with those of Schawalder and Sterchi (11), Bardet et al. (19), Montavon et al. (4), Sumner et al. (33, 38)), Löer (36), Palierne et al. (35), Ginja et al. (23), Palierne et al. (32), and (7) (**Table 1**).

Our findings are inconsistent with (1, 3, 12, 24, 26, 34, 37) (**Table 1**). The use of different measurement techniques can explain the different results of the FAA values. In the current study we found that accurate identification of the sagittal and frontal planes as demonstrated in this study are necessary to delineate the intramedullary axis of the femur. The transverse plane is the appropriate plane to identify the center of the femoral head, the femoral neck axis and the condylar axis to be able to measure the FAA. In addition the size, age, gender, and breed of the dog population also contribute to variations in the FAA (4, 11, 15, 19, 24, 25, 27, 36).

Martins et al. (21) described a significant reduction in FAA in adult animals compared to younger dogs. In contrast, the mean FAA of 7.6° in the Kaiser et al. (24) study, during which magnetic resonance imaging (MRI) was used, is considerably lower than

the mean FAA seen in other studies, this could be due to fact that the femoral head center lies cranially to the plane in which we can define the center of the femoral neck (23, 24).

Some authors confirm a link between an increased anteversion angle and the incidence of degenerative hip diseases such as hip joint dysplasia (1, 2, 4) whilst some others do not (21). This could confirm the high FAA measured by Savio et al. (26) (45°) and by Madsen and Svalastoga (37) (30–43°).

The FAA can support the development of a durable and optimally functional hip prosthesis. The use of correctly designed hip prostheses plays an active role in lowering the risk of postsurgical complications associated with hip arthroplasty in medium and large dog breeds. According to this study, using the methodology described, the measurement of the FAA can be made with good repeatability by a single observer based on using femoral length as a proxy for dog size, a prosthesis FAA of 31 degrees would be suitable for a wide range of dog sizes.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

Ethical review and approval was not required for this animal study as cadavers were obtained from euthanized animals. All research activities were done in accordance with The Central Ethics Committee of Freie Universität Berlin. Written informed consent was obtained from the animal's owner for their use in this study.

AUTHOR CONTRIBUTIONS

All authors have contributed to the conception, design, acquisition of data, analysis and interpretation of data, drafting or revising, and final approval of the manuscript.

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Digital Analysis of Subtrochlear Sclerosis in Elbows Submitted for Dysplasia Screening

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Ulnar trochlear notch (UTN) subchondral bone sclerosis is observed in elbow dysplasia (ED) associated with the medial coronoid disease. However, its evaluation is based on a simple visual examiner assessment of bone radio-opacity level and is considered subjective. The purpose of this study was to objectively characterize the radiographic opacity of the ulnar trochlear notch (UTN) subchondral bone in mediolateral elbow projections classified, using the International Elbow Working Group guidelines. Records and mediolateral flexed elbow images from the Danish Kennel Club database for the ED screening scheme between 2012 and 2018 were available. Of the dogs in the database, those with an ED-negative status in the left limb were identified. From these, 20 dogs each having a status free from ED, or with Grade 1, 2, or 3 in the right limb, were randomly chosen. Joints with primary ununited anconeal process were excluded from the sample. A template was developed using the ImageJ software, for computer UTN sclerosis analysis. It was overlaid onto each image to define five regions of interest (ROIs): ROI-1, distal UTN; ROI-2, middle UTN; ROI-3, caudal UTN; ROI-4, cortical bone; and ROI-5, bone marrow. Mean pixel intensity for each UTN ROI was divided by the mean pixel intensity of ROI-4 to normalize the data. The mean ± standard deviation (SD) of the normalized pixel intensity in the disease joints (ED Grades 1, 2, and 3) was 1.18 ± 0.17, 1.03 ± 0.12, and 0.92 ± 0.09 for ROIs 1, 2, and 3, respectively. The corresponding values for the contralateral normal left joints were 1.16 ± 0.17, 1.01 ± 0.1, and 0.91 ± 0.08. There was a significant difference ($P < 0.05$) in the normalized mean pixel intensity in dysplastic vs. non-dysplastic elbow joints for ROIs 1 and 2. The raw mean pixel intensity from right and left cortical and marrow bone ROIs sometimes showed relatively large differences. Digital radiography is associated with exposure and post-processing variabilities. Differences in apparent radio-opacity (as indicated by pixel intensity) though statistically significant in dysplastic joints compared with contralateral normal joints are slight and are thus problematic for computer-aided assessments of UTN sclerosis.

Keywords: elbow dysplasia, screening, breeding, trabecular pattern, medial coronoid disease

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INTRODUCTION

Elbow dysplasia (ED) in dogs is a developmental hereditary disease, which includes one or more of these primary joint conditions: ununited anconeal process (UAP), osteochondritis dissecans (OCD) of the humeral condyle, medial coronoid disease (MCD), and incongruency of the humero-ulnar joint (1, 2). These conditions result in secondary osteoarthritis leading to pain, discomfort, and lameness (3). The incidence of ED depends on the breed, the population, the screening technique, and the database source, but affected animals can reach up to 70% in some populations (4).

The treatment options for ED are considered relatively limited, and the International Elbow Working Group (IEWG) founded in 1989 recommends radiographic screening of the elbow joint so that dogs with better joint conformation can be selected for breeding. The aim of these schemes is to gradually reduce the prevalence of the disease in canine populations (5). Over the years, the IEWG has developed radiographic diagnostic protocols and scoring system guidelines, which are regularly updated. The IEWG and their guidelines are considered as the international reference among the scientific community for ED screening in most countries concerned with the problem (5–7). However, there are some divergences among national organizations with respect to the type of radiographic projections required for ED scoring. One or more of the following elbow projections may be required: flexed mediolateral, neutral or extended mediolateral, and craniocaudal (or craniocaudal with 15° of pronation). The IEWG ED scoring guidelines recommend the ED scoring as Grade 0 (no signs of arthrosis), ED Grade 1 (mild dysplasia), ED Grade 2 (moderate dysplasia), and ED Grade 3 (severe dysplasia). Evidence of primary elbow disease, as well as the level of osteoarthritic findings, that is, UTN sclerosis and joint osteophytes, is used when determining the ED grade. A borderline sub-scoring between ED Grade 0 and ED Grade 1 is used in some countries (5, 7).

Sclerosis of the UTN is observed in the MCD (8–10). Its evaluation is based on direct visual assessment of bone radio-opacity and is considered to be a subjective parameter, and agreement in its assessment is positively correlated with the radiological experience of the observer (2). This subjectivity is compounded by images with different levels of radiographic exposure, different degrees of joint flexion or other changes associated with the dog's positioning, and variation between breeds. Recently, some studies have been carried out, which associate the increased image pixel values (radio-opacity) of UTN subchondral bone in digital images, evaluated by computerized image analysis, with clinical MCD in dogs (2, 11, 12). However, there are no studies exploring the computerized quantification of UTN sclerosis and relating the degree of sclerosis with ED grades.

The aims of the present study were to characterize the radiographic exposure of digital mediolateral elbow views and to measure the pixel value of the UTN subchondral bone in elbows classified as IEWG ED Grade 0, 1, 2, and 3, using a computer template. The hypothesis of our study was that there is a difference in pixel value of the UTN subchondral bone between ED Grade 0 elbows and dysplastic elbows (ED Grades 1, 2, and 3). The null hypothesis presumed no difference in opacity of the UTN subchondral bone between ED Grade 0 and dysplastic elbows.

MATERIALS AND METHODS

Sample

This was a retrospective study based on the analysis of digital radiographs from the Danish Kennel Club (DKC) database for the ED screening scheme. Records and digital DICOM format flexed mediolateral images in the database for the period between 2012 and 2018 were available. From these records, dogs with a dysplasia-negative status (Grade 0) in the left limb were identified. From these dogs with normal left elbows, four groups of 20 dogs each, having a Grade 0, 1, 2, or 3 in the contralateral right elbow, were randomly selected. This process provided a study set of 80 dogs grouped according to ED grade (left elbow: right elbow) as follows: 0:0, 0:1, 0:2, and 0:3.

Ethical Approval

The protocols were approved by the local Ethics and Administration Committee at the Department of Veterinary Clinical Sciences, University of Copenhagen, and performed in accordance with a Data Sharing Agreement between the DKC and the University of Copenhagen, which in turn complies with the requirements of the General Data Protection Requirements of the European Union.

Computer Sclerosis Analysis

Digital mediolateral elbow views were imported into ImageJ software (version 1.5.3 for Windows) and if necessary rotated so that the proximal radius was positioned horizontally in the image and the cranial part of the humerus orientated to the left (11). The range of pixel value in the images was normalized to 256 shades of gray (0, black; and 255, white) and displayed using a gray scale lookup table typical for radiography (radiolucent areas are relatively dark to areas that are more radiopaque) (11). The approach used was loosely based on published regions of interest (ROIs) of the region (11). This required a user initial input, and then the algorithm creates ROI-1, ROI-2, and ROI-3 for subtrochlear ulnar bone; ROI-4 for cortical bone; and ROI-5 for medullary regions of the ulna. Details of the input and of the created ROIs are shown in **Figure 1**. The macro used for this study and a sample image are included as **Supplementary Material** with the online version of this paper. Mean pixel intensity data from each ROI in the template were measured in ImageJ software and saved as a comma-separated values file.

For further analysis, the pixel intensity of each individual ROI-1, ROI-2, and ROI-3 was divided by the pixel intensity for ROI-4 in the same limb, in order to normalize the data. This step was taken into account for variation in radiographic exposure between images, as described previously (11). The ROI-5 was collected to study pixel intensity cortico-medullar differences in radiographic images. Dogs with evidence of UAP were not included in studies for evaluation of subtrochlear UTN pixel intensity.

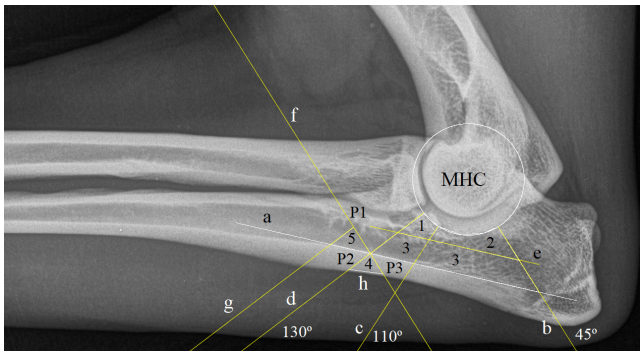

FIGURE 1 | Regions of interest (ROIs) created in ImageJ: A sequence of instructions requiring some user input and performing some logical steps was created as a text file in the ImageJ programming language. For ImageJ, such a list is called a "macro." Line "a" is drawn by the user as a tangent to the caudoproximal endosteal surface of the ulna, and three points are identified by mouse clicks on the edge of the medial humeral condyle (MHC). This user input allows for the automatic creation of the circular outline of the humeral condyle, together with three lines originating in the center of the MHC at angles of 45° "b," 110° "c," and 130° "d" to line "a," a line "e" parallel to line "a" dividing the area bounded by lines "a," "b," and "d" and the humeral condylar, into two regions with areas in the ratio 1 (upper/cranial) to 2 (lower/caudal); line "f" is created again automatically by the macro, perpendicular to line "d" through the point of intersection between lines "d" and "a." Finally, the user marks three points: P1 at the intersection of line "f" with the ulnar cranial endosteal cortex, and P2 and P3 at the points where lines "d" and "f" cross the periosteal caudal ulnar cortex, respectively. This allows the automatic creation of line "g," which is parallel to line "d" and passes through P1, and line "h," which joins P2 and P3. The macro then creates ROIs as follows: ROI-1, distal ulnar trochlear notch (UTN); ROI-2, middle UTN; ROI-3, caudal UTN (single area); ROI-4, cortical bone; ROI-5, bone marrow. The macro takes measurements from these five ROIs. The recorded measurements for each were mean, median, standard deviation, and area. The completed macro sequence thus creates lines and regions according to strict reproducible criteria.

The angles of mediolateral elbow views and the degrees of flexion were measured in the sample using a methodology previously described (13). Details of this measurement are shown in **Figure 2**.

Statistical Analyses

Statistical analysis was performed on the raw and normalized pixel intensity in the different studied groups. The one-sample Kolmogorov–Smirnov test (OSKS) was used to evaluate the normal distribution of variables, and the one-way analysis of variance (ANOVA) and paired *t*-test were used to evaluate if normalized pixel intensity differed significantly between the studied groups. Scatterplot and boxplot graphical analyses were also performed for some of the data studied. The linear Pearson correlation was used to evaluate the association between elbow angles and some of raw and normalized pixel intensity variables. $P < 0.05$ was considered statistically significant. Statistical analysis was performed using the computer software SPSS (SPSS Statistics for Windows Version 27.0, IBM Corp., Armonk, NY, USA).

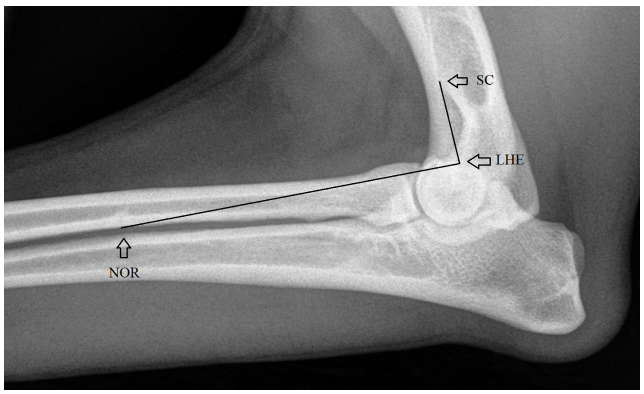

FIGURE 2 | Anatomical landmarks used for measuring the elbow angles, the angular point in the lateral humeral epicondyle (LHE) (contact point of cranial border of medial epicondyle with the condyle) and the linking points at the nutrient orifice of the radius (NOR) and the intersection point of the lateral supracondylar crest and the cranial humeral endosteum (SC) (13).

RESULTS

The age of the 80 dogs used in the sample ranged from 12 to 58 months, mean ± standard deviation (SD) 18.9 ± 9.6 months; and there were 36 males and 44 females. In the sample, there were animals of 22 different breeds; the most common was the German Shepherd, with 20 animals (25%).

The raw mean pixel intensity of cortical and bone marrow ROIs had a normal distribution ($P > 0.05$ in OSKS test). The raw mean pixel intensity of cortical ROI ranged from 90 to 220, mean ± SD, 163.1 ± 27.1; and the bone marrow ROI ranged from 103 to 212, mean ± SD, 168.1 ± 23.9. The differences in raw mean pixel intensity in the cortical minus bone marrow ROIs ranged from −37 to 32, mean ± SD, −5 ± 14.1 (**Figure 3**); those in the right minus left side cortical ROIs ranged from −44 to 45, mean ± SD, 3.2 ± 16.9 (**Figure 4**); and those in the right minus left side bone marrow ROIs ranged from −70 to 49, mean ± SD, 1.7 ± 17.1 (**Figure 5**).

Three dogs with UAP were excluded from ED Grade 3 group. The normalized mean pixel intensity of ROI-1, ROI-2, and ROI-3 also showed a normal distribution in the ED Grade (0, 1, 2, and 3) groups ($P > 0.05$). There was no statistically significant difference in normalized mean pixel intensity in the right ED Groups 0, 1, 2, and 3 in any subtrochlear UTN ROI-1, ROI-2, or 3 ($P > 0.05$, in ANOVA test) (**Table 1**).

The mean pixel intensity of dysplastic right-side elbow joints (ED Grades 1, 2, and 3) were 1.17 ± 0.17, 1.02 ± 0.12, and 0.92 ± 0.09 for ROIs 1, 2, and 3, respectively. The corresponding values for the normal contralateral left joints were 1.14 ± 0.15, 1.00 ± 0.1, and 0.91 ± 0.08. There was a significant difference ($P < 0.05$ in paired *t*-test) in the normalized mean pixel intensity in dysplastic vs. non-dysplastic elbow joints for ROIs 1 and 2 (**Figure 6**).

The elbow angle in mediolateral views ranged from 22.1° to 97.4° (mean ± SD, 57.5° ± 16.6°) and showed some significant Pearson correlations with raw pixel intensity variables

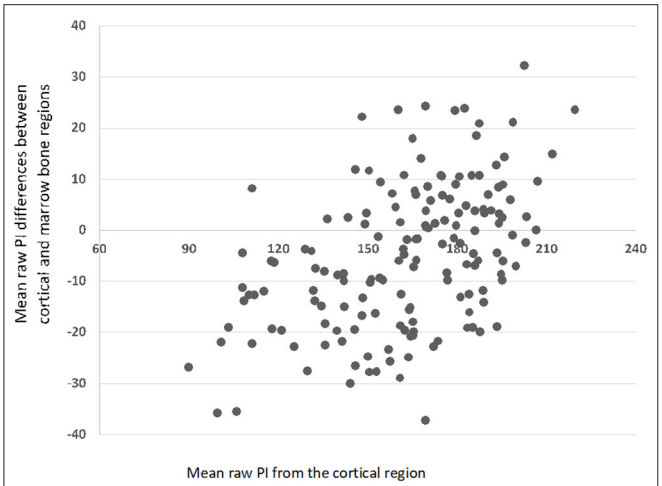

FIGURE 3 | Scatterplot with mean pixel intensity (PI) differences between the cortical and marrow bone regions ($N = 160$).

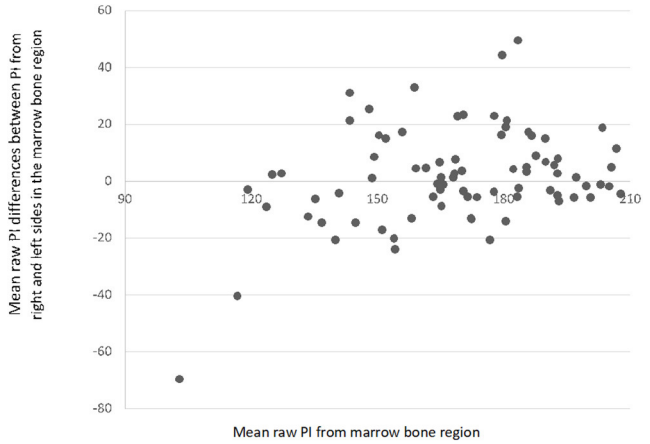

FIGURE 5 | Scatterplot with mean differences between pixel intensity (PI) values from the right and left sides in the medullary region ($N = 80$).

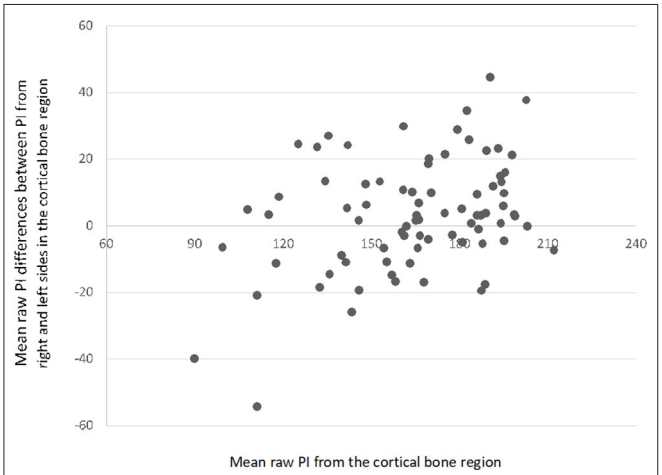

FIGURE 4 | Scatterplot with mean differences between pixel intensity (PI) values from the right and left sides in the cortical bone region ($N = 80$).

(**Table 2**). The association between subtrochlear UTN of raw and normalized pixel intensity ROIs 1, 2, and 3 was also statistically significant ($P < 0.05$) (**Table 2**).

DISCUSSION

The DKC ED screening scheme is based on the IEWG recommendations. The mediolateral elbow flexed view is sent by individual veterinarians to the DKC and ED is evaluated by a panel of scrutineers. Radiographs are scored as Grade 0 (no signs of ED) or Grade 1, 2, or 3 (dysplastic joints), depending on the analysis of the radiographic findings: level of UTN sclerosis, osteophyte size, or signs of primary elbow lesions. Our template was developed on ImageJ software in order to cover the entire UTN subchondral bone, without the overlapping of other bone structures, and to individualize the areas of interest, indicated in previous studies as the most suitable for detecting UTN sclerosis (2, 11, 12).

The random strategy of case selection was to try to eliminate some variability associated with the animal, since it allows the comparison between normal and ED in the same animal using the same imaging equipment and at the same time also allows a comparison between normal and ED in different animals. UAP cases were excluded from the subtrochlear pixel intensity evaluations because in UAP sclerosis is not a common feature and is not used for scoring purposes (14). The results allow us to accept the investigated hypothesis and exclude the null hypothesis for ROI-1 and ROI-2 (most distal and cranial regions of UTN), as the pixel intensity of the UTN subchondral bone in the dysplastic elbows was higher than that of the normal elbow (ED Grade 0) set of contralateral joints. Previous studies had already indicated these anatomic areas as the most predisposed regions for the evidence of UTN sclerosis (2, 11, 12). However, the differences in UTN radio-opacity registered in our research were very small, which did not allow us to recommend this methodology for clinical or ED scoring purposes. This fact is well-demonstrated by the ANOVA test, which did not show significant differences in the comparisons of radio-opacity on right joints scored as Grade 0, 1, 2, and 3 (**Table 1**). Other studies performing similar comparisons, but using only samples of animals affected with MCD and presenting clinical disease, showed evident and statistically significant differences in the subchondral bone UTN radio-opacity (10, 11). Both of these sample factors (MCD and clinical signs) will bias toward the presence of UTN sclerosis. Medial coronoid disease is a primary lesion associated with UTN distal area, as the base of the coronoid process contacts directly with the UTN. In the screening samples of ED control programs, most animals are asymptomatic, so the disease, if present, has not yet reached an advanced stage. Another factor that may have influenced our results is that some ED primary conditions (OCD and incongruency) may promote less subchondral UTN sclerosis than MCD. A previous study

TABLE 1 | Normalized mean pixel intensity (PI) values in the different ulnar trochlear notch (UTN) regions of interest (ROIs) for the right-side elbow joints classified with different elbow dysplasia (ED) grades.

UTN ROI	ED Grade	N	Mean PI values	Standard deviation	Standard error	95% Confidence interval for mean		Minimum value	Maximum value	*P*-value ANOVA
						Lower bound	Upper bound			
1	ED 0	20	1.11	0.11	0.03	1.06	1.16	0.91	1.33	0.55
	ED 1	20	1.18	0.25	0.06	1.07	1.30	1.00	2.01	
	ED 2	20	1.19	0.11	0.03	1.11	1.21	0.96	1.38	
	ED 3	17	1.16	0.12	0.03	1.1	1.22	1.02	1.50	
2	ED 0	20	0.97	0.09	0.02	0.93	1.01	0.82	1.09	0.14
	ED 1	20	1.00	0.15	0.03	0.93	1.07	0.83	1.49	
	ED 2	20	1.04	0.08	0.02	0.99	1.07	0.86	1.19	
	ED 3	17	1.03	0.09	0.02	1.0	1.08	0.94	1.27	
3	ED 0	20	0.91	0.07	0.01	0.88	0.94	0.78	1.05	0.25
	ED 1	20	0.89	0.11	0.03	0.84	0.94	0.66	1.16	
	ED 2	20	0.93	0.07	0.02	0.90	0.96	0.80	1.05	
	ED 3	17	0.94	0.07	0.02	0.90	0.98	0.82	1.10	

ROI-1, distal UTN; ROI-2, middle UTN; ROI-3, caudal UTN.

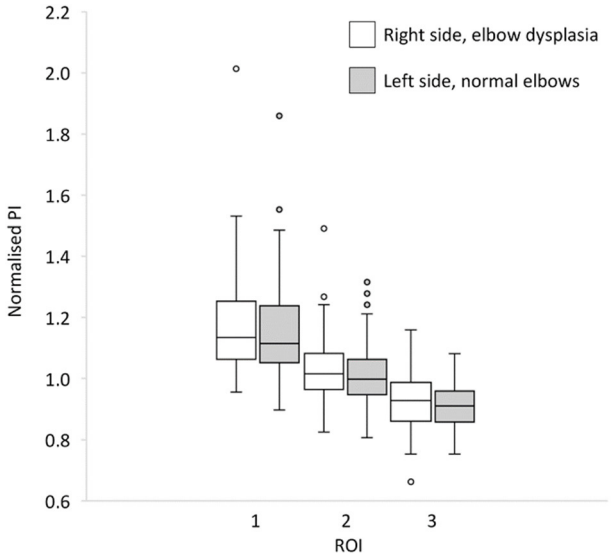

FIGURE 6 | Box-and-whisker plot for comparison between normalized pixel intensity of ulnar trochlear notch (UTN) right-side elbows with elbow dysplasia and contralateral left side normal elbows on regions of interest (ROIs) 1 (distal UTN), 2 (middle UTN), and 3 (caudal UTN) ($N = 60$).

TABLE 2 | Pearson correlations between some of the studied variables: elbow angle (EA), raw (r) pixel intensity of subtrochlear regions of interest (ROIs) 1, 2, 3, 4 (cortex) and 5 (medulla), and normalized (n) subtrochlear nROI-1, 2, and 3.

	rROI-1	rROI-2	rROI-3	rROI-4	rROI-5	nROI-1	nROI-2	nROI-3
EA	0.24*	0.21*	0.21	0.17*	0.18*	0.00	0.04	0.10
rROI-1		0.92*	0.87*	0.71*	0.85*			
rROI-2			0.96*	0.83*	0.89*			
rROI-3				0.89*	0.90*			
rROI-4					0.86*			
nROI-1							0.85*	0.6*
nROI-2								0.84*

**Significant correlation ($P < 0.05$).*

indicated that UTN sclerosis may even be reduced in elbow joints affected only with UAP (14).

The bone density distribution in the subchondral bone plate of the elbow joint of dogs was described using computed tomographic osteoabsorptiometry in normal elbow joints (15). Lower ulnar bone density at the apex of the medial coronoid process with high UTN sclerosis in case of MCD was also described (16). An age-dependent increase in subchondral bone density at the elbow joint, including the UTN, was observed (14, 15). We did not compare the mean pixel intensity between animals with different ages, body weight, conformations, or breeds. We normalized our data to ROIs within the same patient, so any such effects would have been masked.

Our results showed that the evaluation of UTN sclerosis in digital radiographs may become more difficult than in conventional radiographs, since images are actually very different in terms of general radiographic density and contrast. The range of pixel values of cortical and bone marrow among the sample was very large; and sometimes, the mean pixel intensity of the bone marrow ROI-5 was greater than that of the cortical ROI-4 (**Figure 3**). These facts may be related to some breed variability in cortical and bone density, to different parameters used for the radiographic beam (X-ray tube current and voltage, exposure time, and focus–detector distance) during the acquisition of radiographs, but also due to differences in the image processing software used from different manufacturers (17). Even in film radiography, it is established that high kilovoltage techniques will reduce image contrast, and this was likely a cause of variation in our study also (**Figure 3**).

The differences between cortical and bone marrow pixel intensity observed in the contralateral views (right vs. left) are interesting and unexpected (**Figures 4**, **5**). It is likely that imaging software versions, hardware, and beam factors will be the same for contralateral elbows in the same dog. It is accepted however that digital image processing can be influenced by the contents of the field of view, how much of the detector plate is exposed, and the amount and type of tissue and other objects (such as positioning markers), which are included in addition to the target anatomical area. These effects however should be random between the right and left elbows in our study and thus do not explain the difference in pixel intensity seen between the diseased right and normal left elbows. The difference we did see is however unlikely to be clinically useful since ED is often bilateral, and one cannot assume that one will have a normal contralateral limb for comparison.

An additional variability in this study arises from the ED screening images themselves. The elbow angle on the mediolateral views in our sample varied from 22.1° to 97.4° and together with different limb rotations could have interfered with our analysis of UTN sclerosis due to some radiographic summation with bone and soft tissues joint structures. Our results indicate that larger elbow angles are positively associated with more opacity in all raw pixel intensity ROIs (1, 2, 3, 4, and 5) due to soft tissue overlap and opacity summation effects. However, the absence of an elbow flexion angle association with the normalized variables seems to indicate that the interference is eliminated when data normalization is performed (**Table 2**). The significant correlation between subtrochlear ROIs 1, 2, and 3 are expected results, taking into account the anatomical proximity between these regions.

The radiographic image has many optical illusions (18), and one of them is well-demonstrated in this study when assessing cortical bone opacity. Cortical bone may appear to have a uniform and higher opacity than the marrow to the human eye, but in reality, it is non-uniform, with greater bone opacity toward the endosteal surface when compared with the exosteal region. The apparent higher cortical exosteal opacity is due to the optical effects of surrounding lower attenuating soft tissue opacity. Our template evaluates the cortical opacity with a triangular ROI, with its base on the external cortical. It is likely that mean cortical pixel intensity would have been higher had we chosen an ROI that included a more endosteal cortical bone.

Despite the difficulties encountered in identifying ulnar notch sclerosis in this study using objective analysis of digital radiographs, it remains well-established that bone sclerosis is a feature of ED. The mechanism underlying the development of sclerosis is unknown, despite its importance as indicator in the radiographic diagnosis of MCD. It is thought to occur most likely as a result of superimposition of periarticular osteophytosis and an increase in subchondral bone mineral density (10). However, Lau et al. (10) demonstrated the sclerosis of the subchondral UTN bone without interference of periarticular osteophytosis by comparing ulnas with and without sclerosis by computed tomography and radiography. The sclerosis is characterized by the loss of trabecular bone architecture and increased radiographic density. In ED, UTN sclerosis has been linked to increased stiffness of subchondral bone and higher vulnerability of articular cartilage to injury (19). Previous studies concluded that there is a statistically significant association between UTN sclerosis and medial coronoid disease (2, 6, 8, 12), especially when sclerosis is localized in the more distal part of the UTN (11). Quantification of bone density of the medial coronoid process in sound dogs and dogs with fragmented coronoid process (FCP) has also been reported (8).

CONCLUSIONS

The pixel intensity of the UTN subchondral bone in the dysplastic elbow joints was higher compared with that in the normal contralateral elbow joints (ED Grade 0). However, these differences may be more evident if MCD were a feature of ED group. Elbow joint extension is associated with a higher radio-opacity in elbow joint area; however, the normalization of data eliminated this effect in the subchondral UTN region. Digital radiographs are associated with many variabilities due to radiographic parameters and image processing algorithms. These variabilities in pixel intensity make computer quantification of radiographic bone opacity of the UTN in ED difficult and problematic. For visual assessment, it is possible that human image evaluator is prompted by other features, possibly related to trabecular pattern rather than absolute pixel intensity, when concluding on the presence of "bone sclerosis." Further studies are needed to study computer-assisted UTN pixel intensity evaluation using as gold standard not the human visual evaluation but other more accurate tools, like the computed tomography trabecular bone architecture.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

AV: acquisition of data and drafting of the manuscript. SA-P: drafting of the manuscript and critical revision of manuscript. MG: contribution to concept/design, data analysis/interpretation, drafting of the manuscript, and critical revision of manuscript. FM and DN: contribution to concept/design, data analysis/interpretation, and critical revision of manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fvets.2021.664532/full#supplementary-material

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