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PROGNOSTIC CAPACITY OF CURRENT DIAGNOSTIC RADIOGRAPHIC PARAMETERS FOR THE EARLY DIAGNOSIS OF HIP DYSPLASIA IN DOGS: REPEATABILITY AND REPRODUCIBILITY OF LAXITY INDEX MEASUREMENT ON STRESS RADIOGRAPHS OBTAINED WITH THE VEZZONI-MODIFIED BADERTSCHER DISTENSION DEVICE TECHNIQUE IN A LARGE COHORT OF DOGS.

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Summary

Canine hip dysplasia (CHD) is a common development disease leading to secondary osteoarthritis and subsequent clinical signs of discomfort, disability, lameness and pain. Canine hip dysplasia is a complex hereditary, polygenic and multifactorial disease and its early diagnosis allows to offer appropriate conservative or prophylactic surgical treatments in order to reduce the progression of the disease or to discard affected dogs from breading in order to decrease the prevalence of this inherited condition.

Early assessment of CHD includes a careful clinical orthopedic examination and an accurate radiographic evaluation to detect prodromic findings such as hip joint laxity. Stress-radiographs has showed high sensitivity in detecting joint laxity by measuring the distraction index. The PennHIP method is a well standardized, repeatable and reproducible stress-radiographic technique, most popular in the United States. In Europe, the Vezzoni-Modified Badertscher distension device (VMBDD) technique has been proposed as an alternative technique. The reliability of these two methods with very low intra- and inter-observers' variability is paramount to ensure an accurate diagnostic tool for early diagnosis of CHD.

In our study, measurement of laxity index on stress-radiographs obtained with the VMBDD technique revealed to be a repeatable and reproducible procedure only if performed by examiners with moderate or high experience. Nevertheless, the mean differences observed between the measurements obtained by examiners with different experience could be negligible in clinical setting due to the low impact on the definitive diagnosis.

Abstract

TITLE. Prognostic capacity of current diagnostic parameters for the early diagnosis of hip dysplasia in dogs: repeatability and reproducibility of Laxity Index measurement on stress radiographs obtained with the Vezzoni-Modified Badertscher distension device technique in a large cohort of dogs.

Objective: To evaluate the repeatability and reproducibility of the Laxity Index (LI) measured on stress radiographs obtained with the Vezzoni-Modified Badertscher distension device (VMBDD) technique in a large cohort of dogs.
Methods: One-hundred and ninety-five client-owned medium to large breed dogs, aged between 4.5 and 6 months, presented from 2021 to 2024 for screening of hip dysplasia were enrolled in this study. LI for each hip was blindly and independently measured by three observers with different experience on stress radiographs obtained with the VMBDD method. Intra- and inter-observer variability was evaluated. Statistical testing was performed with commercially available software and p-value < .05 was considered significant.
Results: Significant difference between all observers was observed at each measurement session with a Linear Mixed Model and the highest mean difference and its standard error (SE) was 0.074 ± 0.008. No significant difference was recorded between more experienced examiners with the ANOVA test and pairwise t-test. Difference was observed between the first and the second measurement sessions ($p <$ 0.001) and first and the third sessions ($p = 0.001$) in observer 3.
Conclusions: In-house evaluation of the LI on stress-radiographs obtained with the VMBDD technique was a repeatable procedure only if performed by examiners with moderate or high experience, but this could not be claimed for examiners with no or minimal experience. Nevertheless, the mean differences could be negligible in clinical setting due to the low impact on the definitive diagnosis. The LI remained a useful measurement to be used in combination with other clinical and radiographic findings for early diagnosis of CHD.

KEY WORDS: laxity index (LI), repeatability, reproducibility, canine hip dysplasia, stress radiograph.

1. Introduction

Canine hip dysplasia (CHD) is a common development disease of medium, large and giant breed rapidly growing dogs. It is a complex hereditary, polygenic and multifactorial disease, leading to secondary osteoarthritis and subsequent clinical signs of discomfort, disability, lameness and pain [1, 2, 3, 4].

G. Schnelle described the CHD for the first time in the 1935 [5] and several potential causes of the development of the disease have been proposed over the years. The disease is the phenotypic expression of a complex interactions between multiple genes and environmental (no-genetics) factors, such as diet, hormonal changes, rapid growth, body weight and breed [6, 7, 8, 9, 10]. However, the etiology and pathogenesis of the CHD remains still not fully understood.

Henricson *et al*. describe the CHD as a disease that originates from a "vary degree of hip joint laxity, causing subluxation during early life and consequently vary degrees of shallow acetabulum and flattening of the femoral head, inevitably leading to osteoarthritis" [11]. Abnormal joint biomechanics and the resulting inappropriate distribution of the loads result in progressive wearing and erosion of the articular cartilage with exposure and microfractures of the subchondral bone [11, 12]. This condition develops when environmental factors, such as an excessive body weight, an uncontrolled physical activity, or a poor muscle mass, convert the passive laxity to weight-bearing laxity [13, 14, 15, 16].

Radiography is the diagnostic imaging tool commonly used for detection of CHD. Radiographic screenings for CHD are worldwide based on evaluation of the standard ventrodorsal hip-extended (VD) views, according to three international breeding organizations: the Fédération Cynologique Internationale (FCI), the Orthopedic Foundation for Animals (OFA), and the British Veterinary Association and the Kennel Club (BVA/KC). The OFA and the BVA/KC are the screening organizations of United States, and of United Kingdom, Australia and New Zealand, respectively. In Europe, Asia, Russia, and parts of South

America, the CHD is classified according to the FCI five grade scoring system from A, indicating a normal hip joint, to E, indicating severe hip dysplasia [17, 18, 19]. The hip score is subjectively defined on the basis of the joint congruency, the degree of subluxation of the femoral head, the shape and depth of the acetabulum, the shape of femoral head and neck, the presence or absence of signs of osteoarthritis and the measure of the Norberg angle (NA) to quantify the degree of lateral acetabular femoral head coverage [13, 17, 20]. The primary targets for a screening program are to discard affected individuals from the breeding pool and to decrease the prevalence of this inherited condition. In addition, it is also useful for the breeders in order to optimize their financial investments [21].

However, its efficacy to reduce the disease is limited, due to the polygenetic heritable aspect of the CHD [22]: prevalence of CHD is still up to 40% in some breeds despite the intensive screening [17, 23]. Furthermore, this screening method is not suitable for assessing the risk of developing hip dysplasia in young dog. Jassen and Spurrel reported that at 6, 12 and 24 months of age, the 16% to 32%, the 63% to 69% and the 92% to 95% of dogs examined were correctly diagnosed as dysplastic based on the VD radiographic evaluation, respectively [10, 24]. For this reason, the OFA decided to limit the earliest age of canine hip screening to 24 months, while FCI and BVA/KC to 12 months, with a minimum error in diagnosis of 30% [4, 24], with the exception of some dog's breed, for which the minimum age requirement is 15 or 18 months [17].

The CHD is a progressive disease and the assessment of the hips during the growth allows to detect it in its initial development and predict whether or not the dog will develop osteoarthritis. Early diagnosis of CHD is important to raise awareness for the owners about their dog's health and to support any decision about conservative or prophylactic surgical treatments in order to reduce the progression of disease, limiting the development of secondary osteoarthritis [25, 26, 27, 28, 29, 30, 31, 32].

Early diagnosis should be made only when the investigation and skeletal maturity allow it to be reliable. The minimum age required for highly reliable early diagnosis is 4 months in medium and large breeds' dog and 5 months in giant breeds' dog. Before this age the test may have false negative results, except in the rare forms of severe dysplasia [14, 16, 29, 33, 34, 35].

Early diagnosis of CHD is based on a clinical orthopedic evaluation and radiographic examination in static and dynamic views, aimed to detect prodromic findings of the disease, such as hip joint laxity [1, 14]. Hip joint laxity is reported to be a significant predisposing factor for development of hip osteoarthritis and, often, it is the only clinical and/or radiographic findings in dogs predisposed to develop hip dysplasia once skeletally mature [1, 14].

Unlike the VD radiographic view, where the hip extension results in an articular capsule torsion partially hiding the hip laxity [34, 36, 37, 38], the stress-radiographic method has showed high sensitivity in detecting joint laxity [29, 34, 36, 37, 39, 40]. Dorsolateral subluxation scores [39], the subluxation index [40] and the Pennsylvania Hip Improvement Program (PennHIP) method [34] quantify hip laxity radiographically. Over the last 30 years, the PennHIP has been a well-investigated and standardized method [1, 14, 33, 36, 41, 42, 43, 44, 45, 46]. It requires a VD and a compression radiographic view to evaluate the hip congruency and the depth of the acetabulum, and a further distraction view. The latter is performed with the dog in dorsal recumbency with hindlimbs in stance-phase position (± 5°) and the distraction devise placed between them. The device acts as a fulcrum at level of proximal femur and lateralizes the femoral heads when the executor exerts an adduction force [10]. The hip laxity is expressed by quantifying the femur head lateral displacement from the acetabulum, defined as distraction index (DI). The DI is the ratio between the distance of the center of the femoral head and the center of the acetabulum, and the radius of the femoral head [34, 47]. The probability of developing osteoarthritis increases with the increase in joint laxity [48]. Dogs with a DI less than 0.3 are not estimated susceptible to develop CHD and secondary osteoarthritis. Conversely, dogs with an DI higher than 0.3 are considered predisposed [45, 48], with a 100% probability of developing CHD when their DI is greater than 0.7 [49]. When the DI ranges between 0.3 and 0.7 the development of degenerative joint disease is influenced by the muscular conformation of the dog and the environment [29, 49]. A minimal passive hip laxity is physiological and a DI equal to 0 usually indicates an incorrect distraction procedure. A DI value of 1 represents a complete hip subluxation [34]. The PennHIP method is popular in the United States, but it is not widespread in the rest of the world probably due to the costly mandatory training and official PennHIP report, the evaluation fees and the obligation towards digital radiography [33, 50].

The Vezzoni-modified Badertscher distension device (VMBDD) was proposed in Europe as an alternative in-house technique. The VMBDD method was described for the first time by R. Badertscher in 1990 and modified in 1998 by A. Vezzoni [13, 21, 51, 52]. Recent studies have investigated the reliability of this method to assess the hip joint and the interchangeability of the results with the PennHIP method [13, 21, 50, 53]. The stress-radiograph is obtained with the dog in dorsal recumbency and the VMBDD placed between both hindlimbs on the pubic area. Holding both tibia and keeping the femurs in minimal extension, 95-105° in respect to the vertebral column (or ± 10° extension relative to the neutral standing position), a medial compression is applied in order to force the femurs against the distractor device permitting lateralization and subluxation of the femoral heads [21]. Likewise for the PennHIP method, also for the VMBDD technique the DI is measured in a similar manner described above, but the authors used the term Laxity Index (LI) in order to distinguish it from the DI [13, 50, 53, 54, 55]. The LI obtained with the VMBDD technique yields results similar to the PennHIP-based DI and the interobserver agreements of the DI and the LI are similar [50].

The scientific literature reports a satisfactory technical repeatability and reproducibility of the VMBDD technique and recommends it as a reliable in-house evaluation method of the hip joints in young patients, with a quick and easy learning curve for inexperienced examiners [13, 53]. Authors report a high inter-observer and intra-observer agreement for LI measurement in a closed cohort of dogs, where numerous observers were involved [53, 56]. However, the measurements were performed in one study in only 10 dogs of different breeds and with an age ranging between 11 months and 1 year and 4 months old [53]; and in another study in a closed cohort of four-month-old Rottweilers [56].

The performance of these assessments in a larger group of dogs of different breeds and with a limited age range, has been not tested yet.
The aim of our study is two-fold: a) to evaluate the intra- and inter-observer variability of the LI measurements in a large cohort of different breed dogs in order to evaluate its repeatability and reproducibility, respectively; b) to assess the influence of experience in performing LI measurements by involving observers with a different knowledge background and experience. A larger population would allow us to perform a more appropriate statistical analysis and eventually detect different results from those reported so far in the literature.

2. Materials and Methods

2.1. Animals

Medium to large breed dogs, aged between 4.5 to 6 months, referred to Veterinary Teaching Hospital of the University of Camerino (Matelica, Macerata, Italy) and Veterinary Clinic San Silvestro (Castiglion Fiorentino, Arezzo, Italy) between November 2021 and November 2024 for screening of hip dysplasia, was enrolled in this study.
Age, gender, breed, body weight (BW) and body condition score (BCS, 1 to 9) were recorded. All the patients underwent complete orthopedic examination. After an anaesthetic examination and blood tests confirming unremarkable results, the dogs were premedicated with 3 μg/kg of dexmedetomidine and 0.2 mg/kg of methadone intramuscular (IM) and induced with 1–4 mg/kg of propofol intravenously (IV) to effect until tracheal intubation. Anesthesia was maintained with 1.2% isoflurane in 100% oxygen during all the radiographic evaluation. The radiographic examination includes four radiographic views of the pelvis: a VD, a frog leg, a dorsal acetabular rim (DAR) and stress radiograph views [29, 21, 57].
For our study, only the stress radiographic views were blindly evaluated.

2.2. Stress radiograph acquisition and LI measurement

The stress radiographs were performed by orthopedic surgeons with 3 to 20 years of experience with the VMBDD technique. Despite the experience with the device, the surgeons attended a theoretical-practical course organized by Veterinary Teaching Hospital of the University of Camerino and Veterinary Clinic San Silvestro to standardize the stress radiographs technique according to the literature [13, 21, 52].

The VMBDD device has a trapezoidal-shaped Teflon structure with a mild S-shaped curvature to adapt to the pubic area and with a base articulated to a parallelepiped Teflon structure to keep parallel to the table [21], equipped with non-slip rubber feet. The trapezoidal-shaped structure has an 8 x 8 cm hole to accommodate the patient's tail, it is 50 cm long, 2 cm high, and 12 to 6 cm wide. The parallelepiped structure is 6 cm long, 2 cm high, and 50 cm wide ► **Figure 1**.

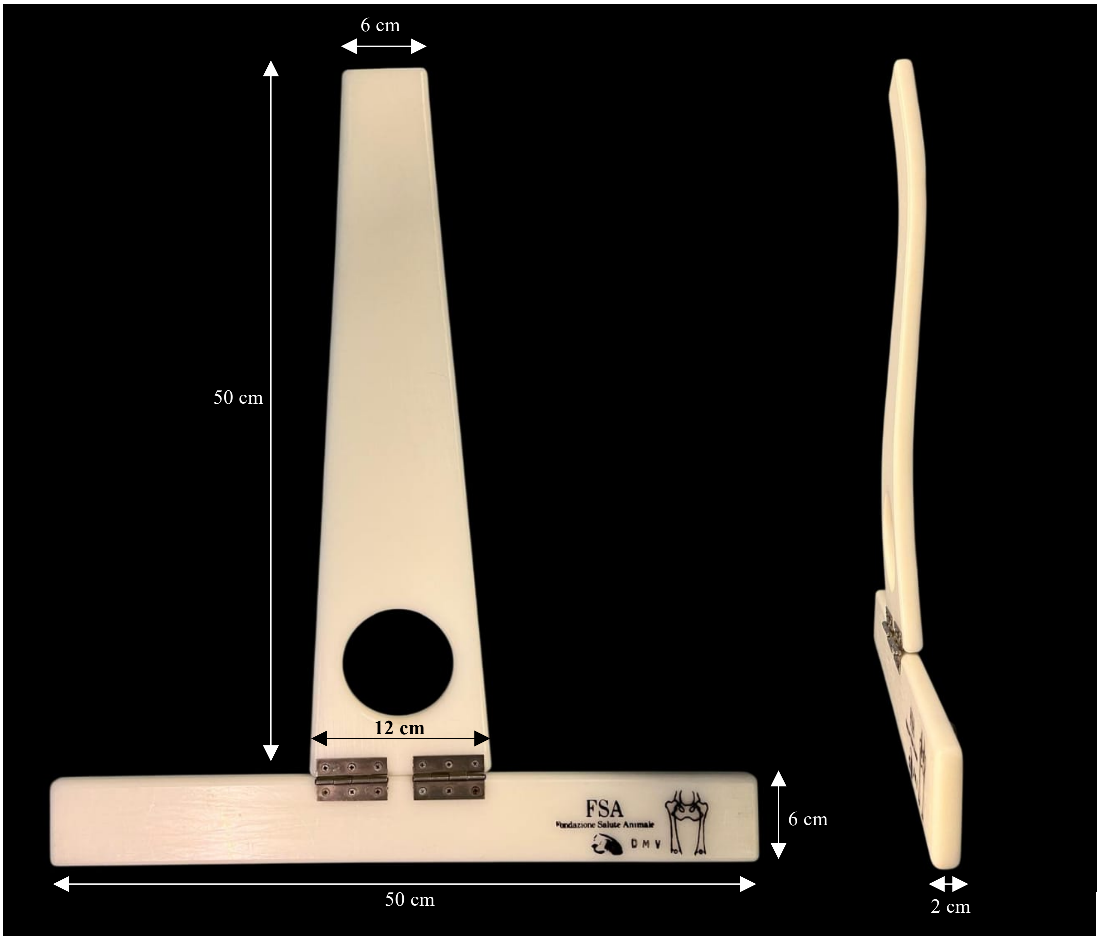

Figure 1. Vezzoni-modified Badertscher distension device (VMBDD).

The stress radiographs with the VMBDD distractor were obtained as previously described [13, 21, 52]. Briefly: the dogs were positioned in dorsal recumbency and the VMBDD placed between both hindlimbs on the pubic area, with the lateral margins of the distractor in contact with the inner surface of the dog's thigh. Holding both tibia in a horizontal position and the femurs in minimal extension to avoid superimposition of the stifle joint over the hip (95-105° with respect to the vertebral column or ± 10° extension relative to the neutral standing position), a medial compression was applied to force the femurs against the distractor device permitting lateral subluxation of the femoral heads The medial compression was synchronous with the delivery of the X-ray beam [21]. ► **Figure 2**.

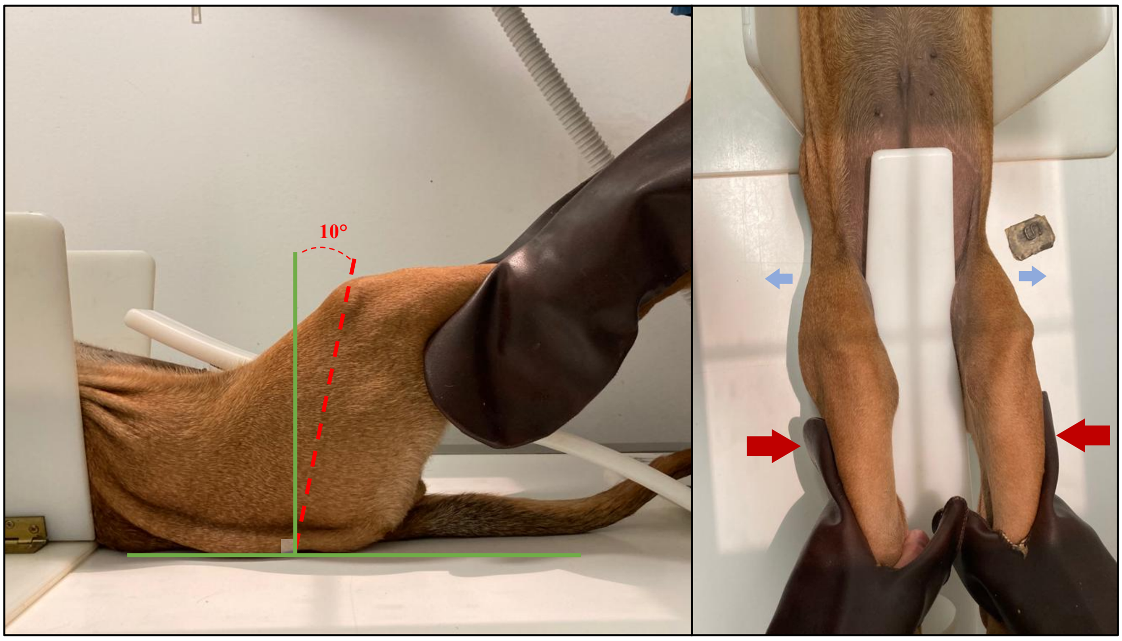

Figure 2. Stress radiograph performed with Vezzoni-modified Badertscher distension device (VMBDD) technique. The distractor device was placed between both hindlimbs on the pubic area, both tibiae kept in a horizontal position and the femurs in ± 10° extension relative to the neutral standing position. The femurs were forced against the device applying a medial compression (red arrows) and permitting lateralization and subluxation of the femoral heads (blue arrows).

Correct positioning and adequate radiographic image ensured a straight and symmetrical pelvis (symmetrical obturator foramina and iliac wings), not superimposition of the stifle joint over the hip, symmetric femurs, and a lateral displacement of the femoral head in comparison to the VD view ► **Figure 3a**. The DICOM radiographic images were assessed with an open-source medical image viewer (Horos, DICOM viewer, version 3.3.6, Horos Project) and the LI was measured as previously described. The LI was obtained by outlining the acetabulum and the head of the femur with a circle and measuring the distance (d) between the geometric centers of these two circles (the geometric center of the acetabulum and the geometric centers of the femoral head). The LI was calculated by dividing the distance (d) with the radius of the circle bounding the femoral head (r): LI = d/r ► **Figure 3b** [29, 21, 34, 50, 53, 57].

The LI measurements were blindly performed by three observers with different experience in veterinary orthopedics and in hip dysplasia disease and all data were recorded using commercial software (Microsoft Excel, Version 16.92, ©2024 Microsoft). The observers were a senior orthopaedic surgeon (APP) with more than 20 years of practice and a PhD in Orthopaedics (observer 1), a PhD student in Orthopaedics (SS) with 4 years of practice (observer 2) and a student of veterinary medicine (EC) (observer 3). The observer 3 has never performed laxity measurements previously. Therefore, it was mentored by the observers 1 and 2, who showed and taught it in detail all the steps to measure the laxity index.

Each observer blindly measured the LI three times (three measurement sessions) on each hip, with a washout period of two weeks between measurement rounds. All measurements were obtained by each observer in about six weeks. The left and the right hips were evaluated separately. Before the three measurement sessions, each observer performed independently test measurements on 10 hip joints in order to train. The test measurements of 10 hip joints were not recorded and were not considered for the study.

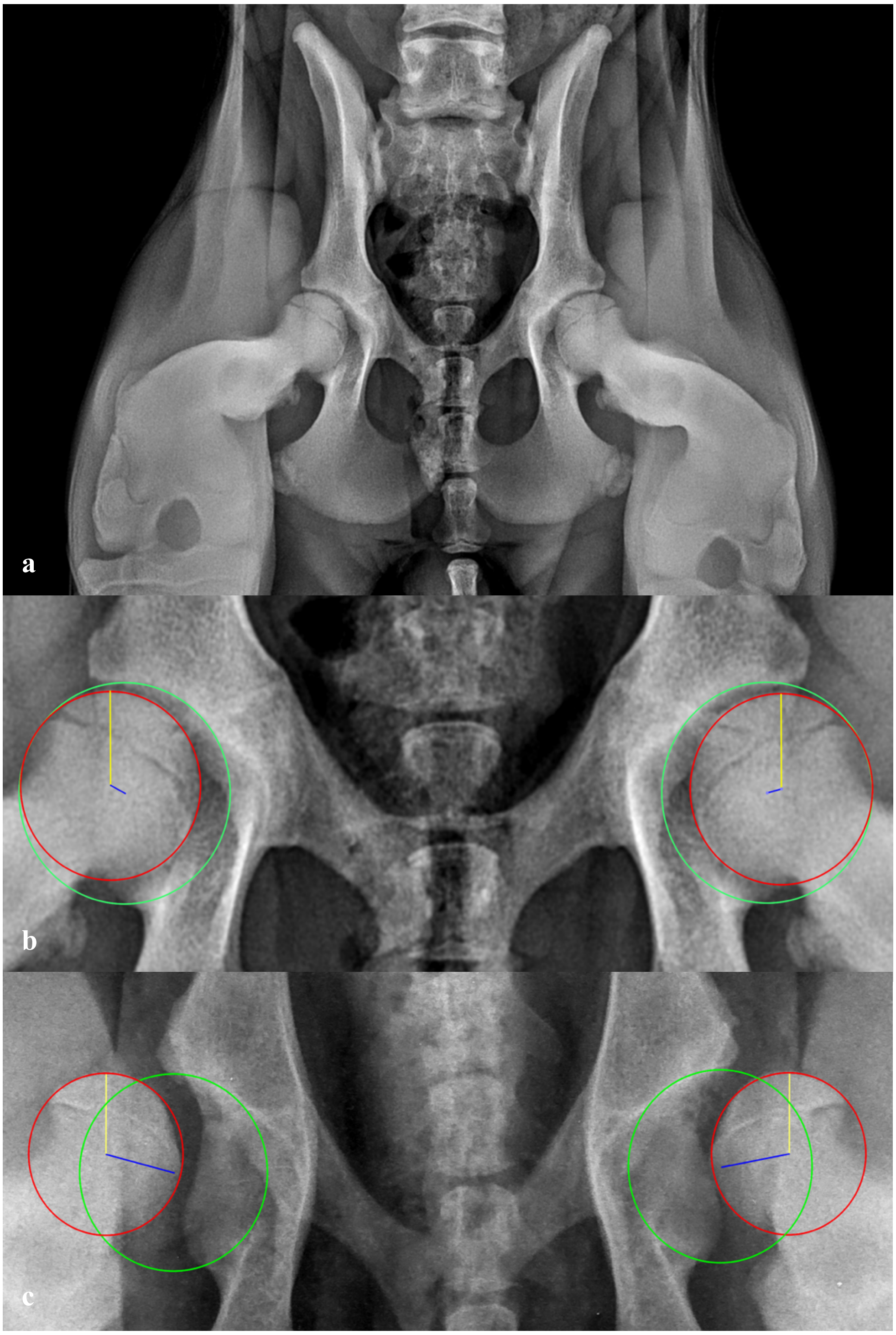

Figure 3. **a)** Correct stress radiographic view. The obturator foramina and iliac wings are symmetrical ensuring a straight pelvis. The femurs are symmetrical and the femoral head laterally displaced. **b)** Laxity Index (LI) measurement in a patient with IL < 0.3. The acetabulum was delineated with a circle passing through the craniolateral acetabular edge, the craniomedial acetabular edge and the caudolateral acetabular edge (green circle). Secondly, the head of the femur was delineated with a circle passing through the cranial and caudal aspects of the head (red circle). Finally, the distance (d, blue line) between the centers of the acetabulum circle and the femoral head circle was measured and the LI was calculated (LI = d/r), where "r" was the radius of the circle delimiting the femoral head (yellow line). **c)** LI measurement in a patient with IL > 0.7.

2.3. Statistical Analysis

Continuous variables were reported as either means and standard deviation (SD) or median and interquartile ranges (IQRs) according to their distribution, as assessed by the Shapiro-Wilk normality test. Categorical variables were reported as absolute frequencies and percentages. Linear Mixed Models (LMM) were performed to estimate the covariates longitudinal effect on the continuous LI scale. Liner Mixed effects regression model uses all available data and can properly account for correlation between repeated measures. The covariates included in the model were the observer (i.e., three different observers) and the time-point as categorical variable and their interaction. For groups effect testing, the Tukey post-hoc test was performed. For multiple comparison within time point and between observer groups, ANOVA test was performed and pairwise t-test with p-value adjusted by Holm approach. Whereas, for multiple comparison within observer groups and between time-points, repeated measures ANOVA test was performed and pairwise t-test with p-value adjusted by Holm approach. All the statistical values equal to or smaller the 0.05 were considered statistically significant. The analysis was conducted using the R statistical software (version 4.1.3; 10-03-2022). The calculated LI were subdivided into breeds for evaluation of breed influence and the statistical analysis was repeated in subgroups with more than 20 dogs.

3. Results

One-hundred and ninety-five client-owned dogs were enrolled in this study. There were one-hundred and eight males and eighty-seven females (55.4 % males and 44.6% females). Their mean ± SD age was 5.2 ± 0.6 months and their mean ± SD BW was 18.6 ± 5.8 kg, with a mean ± SD BCS of 4.4 ± 0.7 (median, min-max; 4, 3 – 7).

Thirty-nine breeds were represented: 33 Golden Retriever, 30 Border Collie, 29 Labrador Retriever, 21 German Shepherd, 17 mixed breed dog, 8 Bernese Mountain dog, 8 Australian Shepherd, 5 Cocker Spaniel, 5 Cane Corso, 4 Maremma Sheepdog, 3 Dobermann, 3 Rottweiler, 2 English Setter, 2 American Staffordshire Terrier, 1American Bully, 1 American Pitbull Terrier, 1 Siberian Husky, 1 Hungarian hound, 1 Bassethound, 1 Bobtail, 1 Czechoslovak Wolf, 1 Kelpie, 1 Apuan Shepherd dog, 1 Shiba Inu, 1 Springer Spaniel, 1 Rhodesian Ridgeback, 1 Pyrenean Mountain dog, 1 Great Dane, 1 Newfoundland dog, 1 Romanian Shepherd, 1 Neapolitan Mastiff, 1 Belgian Shepherd Malinois, 1 Alaskan Malamute, 1 British Staffordshire Terrier, 1 Nova Scotia Retriever, 1 Weimaraner, 1 Lagotto Romagnolo dog, 1 Bavarian Hound and 1 Caucasian Shepherd. Three-hundred and ninety hip joints were analyzed (195 left hip joints and 195 right hip joints).

The mean ± SD of LI measurement obtained by the three observers are shown in ► **Table 1**. The measurement was not affected by the joint side: the LI had the same stratification for measurements performed in left and right hip joint ► **Figure 4.**

Table 1. Mean and standard deviation of LI measurement.

LI	T1	T2	T3
Observer 1	0.41 ± 0.15	0.41 ± 0.16	0.41 ± 0.15
Observer 2	0.44 ± 0.23	0.43 ± 0.24	0.44 ± 0.23
Observer 3	0.48 ± 0.17	0.45 ± 0.17	0.45 ± 0.17

Abbreviations: LI, laxity index; T1, 1° measurement session; T2, 2° measurement session; T3, 3° measurement

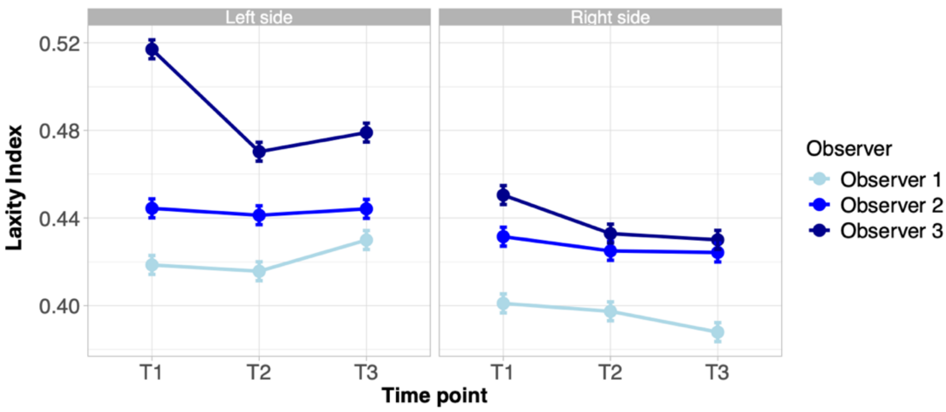

Figure 4. Laxity Index trends across observers and time-points (measurement sessions) stratified for left and right sides.

The ANOVA test and pairwise t-test with Holm's approach for p-values adjustment revealed significant difference between observer 1 and observer 3 (T1, $p < 0.0001$; T2, $p < 0.0001$; T3, $p = 0.0001$), and observer 2 and observer 3 (T1, $p < 0.0001$; T2, $p = 0.01$; T3, $p = 0.01$), at each measurement session. No significant difference was recorded between examiners 1 and 2 ► **Figure 5**. In observer 3 the measurements were significant different between the first and the second measurement sessions, and the first and the third sessions ► **Figure 6**.

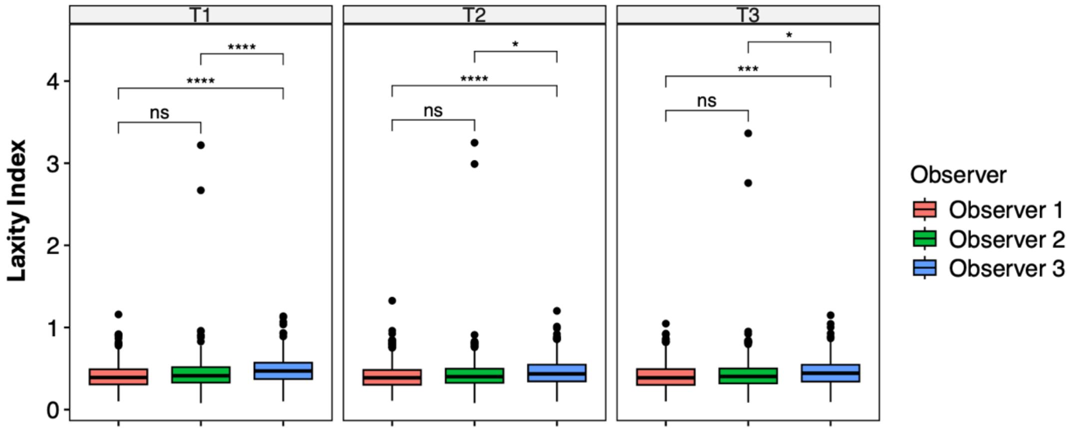

Figure 5. Laxity Index distributions across observers' groups intra time-points. Means difference were tested by pairwise t-test with Holm's approach for p-values adjustment. Asterisk (*) indicates a significant difference (p < 0.05), in particular: ****(p < 0.0001), ***(p = 0.0001), **(p = 0.001), *(p = 0.01), ns (p = 0.05 or p > 0.05).

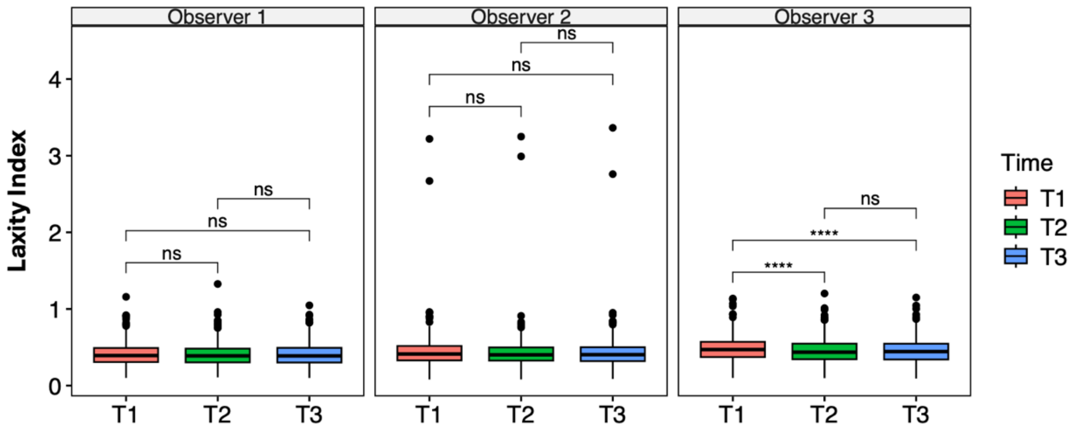

Figure 6. Laxity Index distributions across time-points intra observers' groups. Means difference were tested by pairwise t-test for paired samples with Holm's approach for p-values adjustment. Asterisk (*) indicates a significant difference (p < 0.05), in particular: ****(p < 0.0001), ***(p = 0.0001), **(p = 0.001), *(p = 0.01), ns (p = 0.05 or p > 0.05).

The LMM partially confirmed the results of ANOVA test and pairwise t-test with Holm's approach for p-values adjustment. The LMM showed significant difference between all observers at each measurement session and the highest mean difference and its standard error (SE) between observers was 0.074 ± 0.008. Only operator 2 and 3 did not differ significantly during the second round of measurements ► **Table 2**, **Figure 7**.

Table 2. Means difference of Laxity Index between observer groups intra-time obtained by linear mixed model. *P*-value adjustment by Tukey approach for comparing a family of three estimates.

		Mean difference estimate	**SE**	**p-value**
T1	Obs. 3 – Obs. 1	0.074	0.008	**< 0.0001**
	Obs. 3 – Obs. 2	0.046	0.008	**< 0.0001**
	Obs. 1 – Obs. 2	-0.028	0.008	**0.002**
T2	Obs. 3 – Obs. 1	0.045	0.008	**< 0.0001**
	Obs. 3 – Obs. 2	0.019	0.008	0.069
	Obs. 1 – Obs. 2	-0.027	0.008	**0.004**
T3	Obs. 3 – Obs. 1	0.046	0.008	**< 0.0001**
	Obs. 3 – Obs. 2	0.020	0.008	**0.040**
	Obs. 1 – Obs. 2	-0.025	0.008	**0.007**

Abbreviations: T1, 1° measurement session; T2, 2° measurement session; T3, 3° measurement session; Obs., Observer; SE, Standard Error.

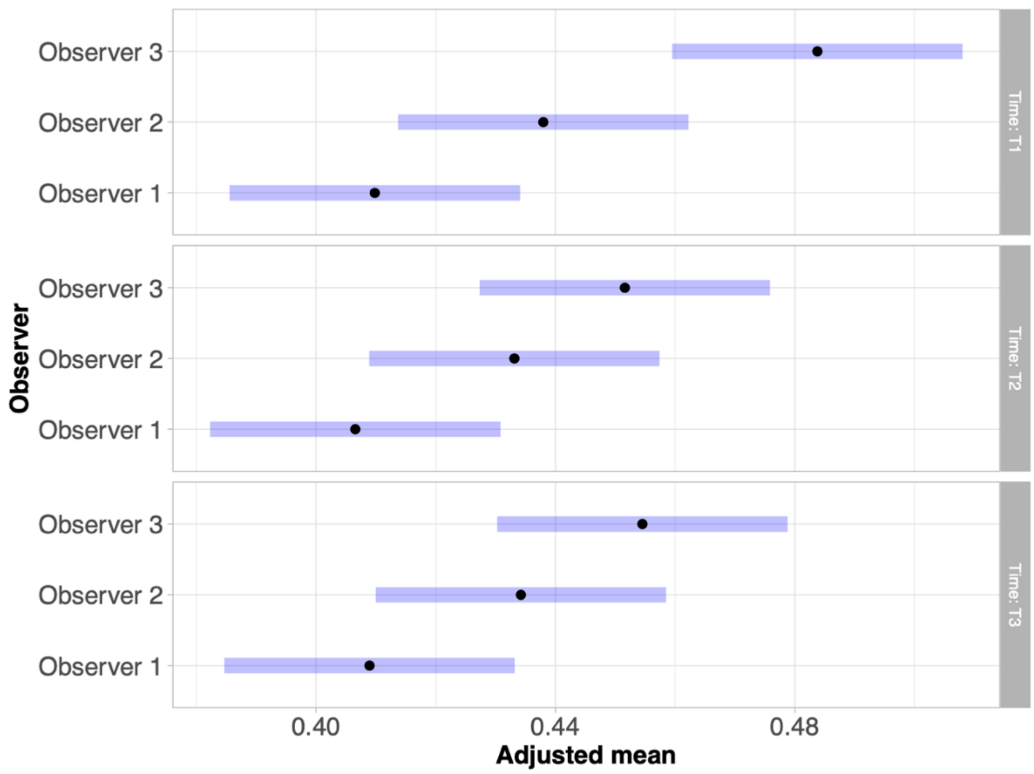

Figure 7. Adjusted means of Laxity Index across observer groups intra time-points.

The significant difference was observed between the first and the second measurement sessions ($p < 0.001$) and first and the third sessions ($p = 0.001$) in observer 3 by the LMM, agreeing with ANOVA test and pairwise t-test ► **Figure 8**. The mean difference ± SE was 0.032 ± 0.008 between T1 and T2 and 0.029 ± 0.008 between T1 and T3.

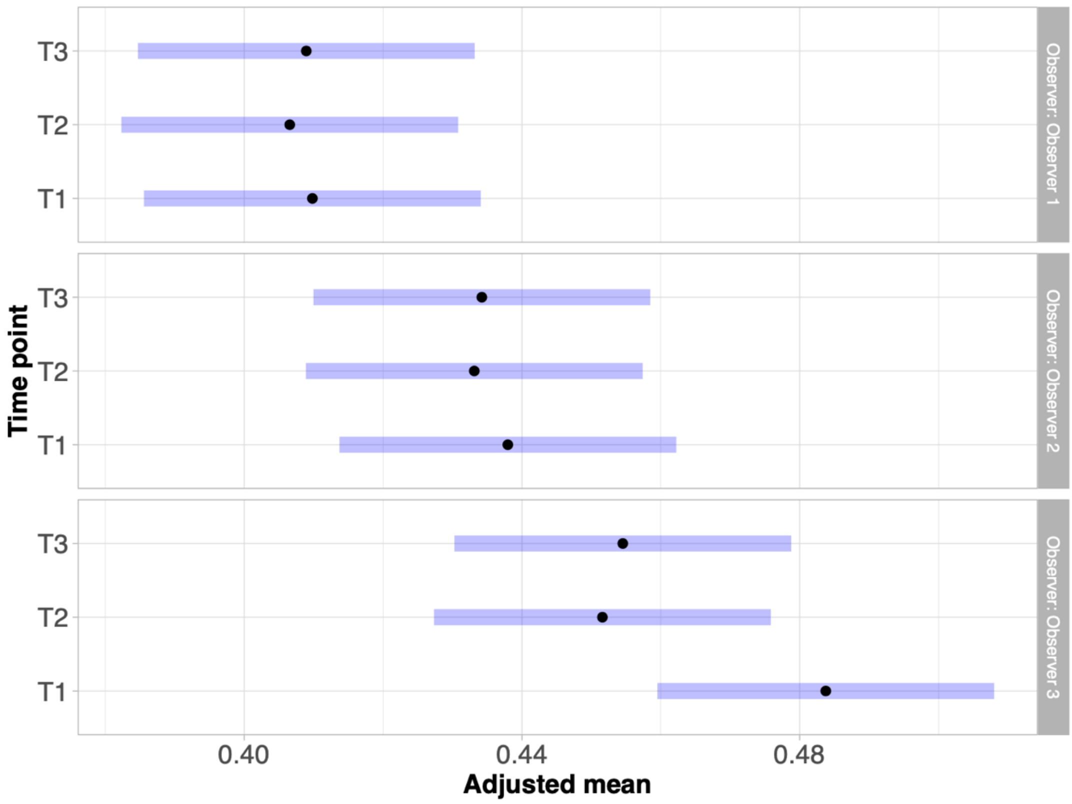

Figure 8. Adjusted means of Distraction index across time-points intra observer groups.

Dividing the study population into breeds, 4 subgroups of more than 20 dogs were identified: Golden Retriever (GR) group, Border Collie (BC) group, Labrador Retriever (LR) group and German Shepherd (GS) group. The mean ± SD age, BW, BCS and LI, and the gender of subgroup are shown in ► **Table 3**.

Table 3. Mean and standard deviation (SD) of age and LI of difference of GR, BC, LR and GS subgroups.

	GR group	**BC group**	**LR group**	**GS group**
Age (months)	5.1 ± 0.6	5.1 ± 0.4	5.3 ± 0.7	5.0 ± 0.5
BW (kg)	20.1 ± 3.9	13.1 ± 3.2	19.5 ± 3.2	20.6 ± 3.0
BCS	4.6 ± 0.6	4.1 ± 0.4	4.8 ± 0.7	4.1 ± 0.5
Gender	17 M, 16 F	17 M, 13 F	15 M, 14 F	10 M, 11 F
LI	0.47 ± 0.13	0.38 ± 0.31	0.42 ± 0.16	0.40 ± 0.11

Abbreviations: BW, body weight; BCS, Body Condition Score; LI, laxity index; GR, Golden Retriever; BC, Border Collie; LR, Labrador Retriever; GS, German Shepherd.

The results of ANOVA test and pairwise t-test performed to analyze data between time points for each observer in each breed subgroup agreed with the analysis previously performed on the entire population. The LI of observer 3 showed statistically significant difference between first and second measurement sessions in all breed group (GR group, $p = 0.0001$; BC group, $p = 0.001$; LR group, $p < 0.0001$; GS group, $p = 0.01$) and between first and third session in BC ($p < 0.0001$) and LR group ($p = 0.0001$). In GR group, also the observer 2 showed a statistic difference between T1 and T2 ($p = 0.01$) ► **Figure 9**. However, during the first measurements round, significant differences were recorded between observer 1 and 3 in GR, LR, GS groups ($p = 0.001$) and slight significant differences between observer 2 and 3 in GR and GS groups ($p = 0.01$). During the last measurements session slight significant differences were revealed between observer 1 and 3 only in GR and GS groups ($p = 0.01$) ► **Figure 10**.

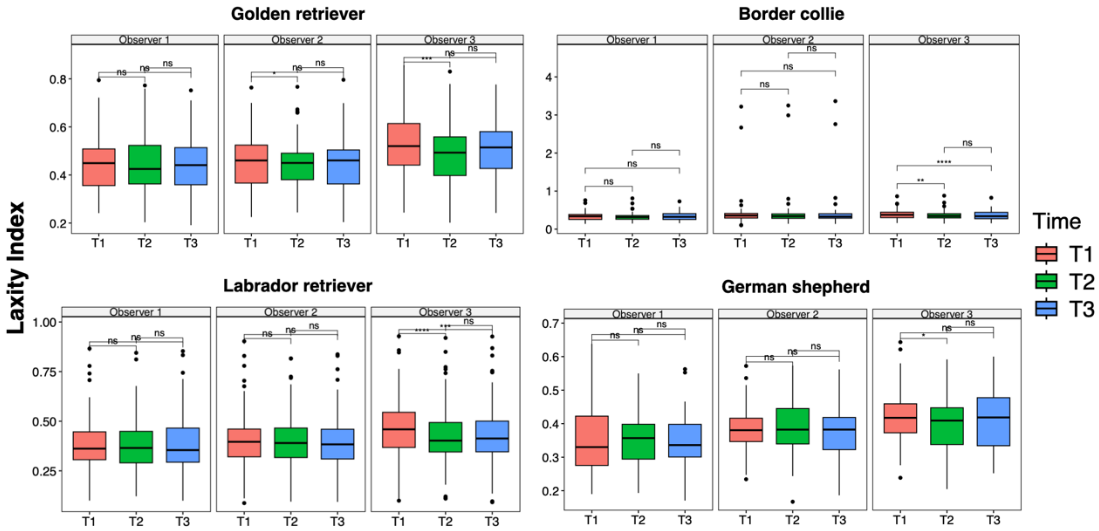

Figure 9. Laxity index distributions across time-points intra observers' groups in Golden retriever, Border collie, Labrador retriever and German shepherd subgroups. Means difference were tested by pairwise t-test for paired samples with Holm's approach for p-values adjustment. Asterisk (*) indicates a significant difference ($p < 0.05$), in particular: ****($p < 0.0001$), ***($p = 0.0001$), **($p = 0.001$), *($p = 0.01$), ns ($p = 0.05$ or $p > 0.05$).

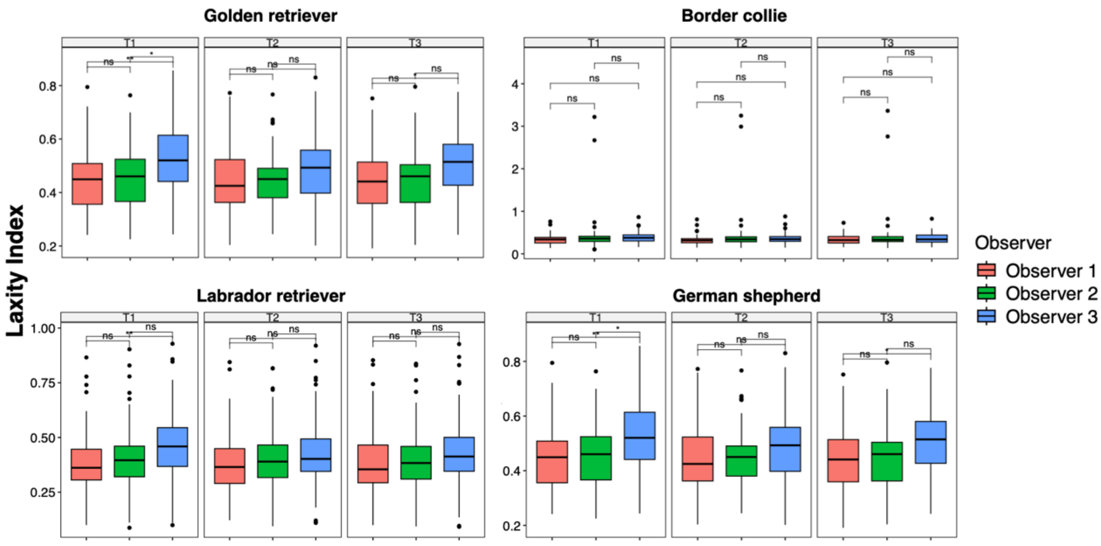

Figure 10. Laxity index distributions across observers' groups intra time-points in Golden retriever, Border collie, Labrador retriever and German shepherd subgroups. Means difference were tested by pairwise t-test for paired samples with Holm's approach for p-values adjustment. Asterisk (*) indicates a significant difference ($p < 0.05$), in particular: ****($p < 0.0001$), ***($p = 0.0001$), **($p = 0.001$), *($p = 0.01$), ns ($p = 0.05$ or $p > 0.05$).

4. Discussion

The aim of our study was to assess the intra- and inter-observer variability of the LI measurements in a large cohort of dogs of different breeds in order to evaluate the repeatability and reproducibility of the LI. Although the literature has already evaluated the intra-observer and inter-observer variability of LI measurements with successful results [50, 53, 54], our data, obtained from a large cohort of different breed dogs, showed a significant inter-observer variability. This difference was detectable, albeit to a lesser extent, between the senior orthopaedic surgeon (observer 1) and the PhD student (observer 2) with a maximum value of the mean difference of 0.028 ± 0.008. This difference was only detected by the LMM, which considers the interaction between the variable "observer" and the variable "time", but it was not detected by ANOVA test and pairwise t-test. Furthermore, maximum value of the mean difference between observer 1 and 2 was such that it did not affect the definitive diagnosis of CHD. Literature reported that hip joints with DI less than 0.4 were not predisposed to develop CHD, while hip joints with DI higher than 0,7 developed moderate to severe CHD [21]. There were doubts about patients with DI between 0.4 and 0.7, who had a different evolution depending on age, joint [21] and muscular conformation of the dog and the environment [29, 39, 49]. Considering this range of value, a DI that varies ± 0.028 should not bring any change in the interpretation of the data.

The two performed statistical tests agreed on the statistically significant difference between the LI measured by observer 3 and those measured by observer 1 and 2. The student of veterinary medicine (observer 3) performed the LI measurement for the first time during this study, after a personal study and didactic support. It was therefore the operator with less experience. We were expecting statistically significant difference compared to other operators. So, our result differed statistically from what was previously published [50, 53, 56], but, despite the statistically significant difference, the higher mean difference and its standard error between observer 1 and the others was of 0.074

± 0.008. This value could be negligible due to the low impact on the definitive diagnosis because the difference in clinical practice would be not relevant.

Vidoni et al. reported excellent to good inter-observer agreements for quantitative values such as Norberg Angle (NA), Dorsal Acetabular Rim slope (DAR), Center Edge Angle (CEA) and LI, but the high level of experience of their observers ensured the accuracy of the measurements [56]. Our study consisted of a more heterogeneous group of observers, with different levels of experience, who led to clinically negligible but statistically significant difference. The experience of the examiners could affect the result and the inter-observer agreement could increase with the experience [20]. To support this statement, our results reveal intra-observer variability between the LI measured by the less experience operator during the first measurement session and the others time points. During the next two rounds, the observer 3 increase the intra-observer agreement, also reducing the inter-observer variability: it reduced the amount of the significant difference between operator 2 and 3 so that no difference is detected between these operators at second time-point (T1 $p < 0.0001$ versus T2 $p = 0.069$ and T3 $p = 0.040$). Nevertheless, even in this case, the maximum mean difference was such that it would not affect the diagnosis in clinical practice (0.032 ± 0.008). Our results did not reveal intra-observer variability between the LI measured and calculated by the most experienced operators. During each measurement session, the senior orthopaedic surgeon (observer 1) and the PhD student (observer 2) were consistent with their results, emphasizing the repeatability of the LI measured by operators with more experience.

Our study was conducted on a higher number of cases in comparison to published studies, [50, 53, 56] in order to perform a more appropriate statistical analysis and eventually detect previously undetected variability. This allowed us to note that the inexperience of the operator affected the measurements performed and a moderate or high experienced examiner (PhD student in Orthopaedics and senior orthopaedic surgeon, respectively) guarantee more accurate measurements. Nevertheless, it is necessary to be aware that there was a statistic difference between all the operators, even if the mean difference did not influence the prognosis.

The high number of patients enrolled in the study allowed us to further investigate variability of these measurements considering the different breeds present. Statistical analysis performed in the breed subgroups reflected the statistical analysis executed on the entire cohort. It was interesting to note that during each measurement session, the observers did not show statistically significant differences in the subgroup of Border Collies, confirming high repeatability. The absence of inter-observer variability in the Border Collie subgroup could indicate the presence of more defined anatomical landmarks in that breed. The remaining sub-groups partly reflected the results of the general statistical analysis.

Our examiners had difficulties in some dogs to outline the acetabular cavity, in particular to detect the medial end of the cranial acetabular margin and the caudal pillar of the acetabulum, increasing the risk of intra- and inter-observer variability. These anatomical landmarks affect the position of the center of the acetabular cavity and therefore the distance between it and the center of the femoral head. Difficulties in delineating the acetabulum and the femoral head were previously reported [53, 58]. Further studies are needed to identify possible anatomical differences of the coxo-femoral joint in different breeds, in both adult and skeletally immature dogs. These anatomical studies could be interesting not only for the purpose of carrying out these measurements, but also to plan the best therapeutic procedures (such as radiographic planning of the acetabular cup for a total hip replacement) [59, 60, 61]. From the data we collected, we couldn't state that there were structural differences about the anatomical landmarks needed for the calculation of the DI relative to breed, but there were individual differences that could make these points more or less evident ►**Figure 11**.

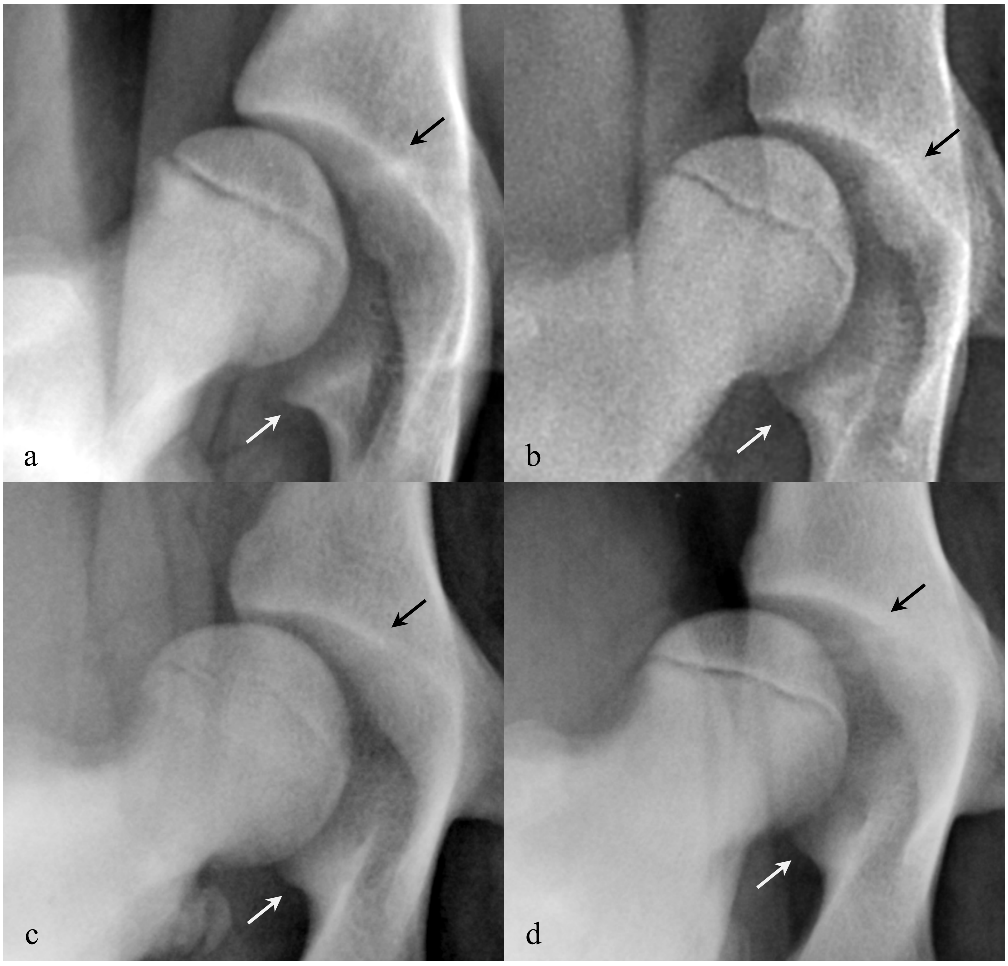

Figure 11. **a)** Distracted hip joint of a 4,5 months old, female, German shepherd. **b)** Distracted hip joint of a 5 months old, female, German shepherd. **c)** Distracted hip joint of a 6 months old, male, Labrador retriever. **d)** Distracted hip joint of a 5 months old, male, Labrador retriever. The image **b-d** shows a caudal pillar (white arrows) slightly-moderate less pronounced than the image **a-c**. The image **c-d** shows a craniomedial acetabular edge (black arrows) more defined than the image **a-b**.

In addition, we did not investigate the influence of age on the detection of anatomical landmarks because we enrolled patients with an age ranging from 18 to 24 weeks. It is recommended to perform the radiographic study during this age range for early diagnosis of hip dysplasia, due to the developmental nature of this disease: the anatomical elements evaluated during the radiographic examination for early diagnosis of CHD could be more difficult and uncertain to evaluate before17 weeks of age [21] and a diagnosis after the 24 weeks of age could be not useful for some surgical treatment (Juvenile Pelvic Symphiodesis) to reduce the progression of disease [25, 26, 27, 28, 29, 30]. However, considering that the articular surfaces and joint laxity can change with the growth, it would be interesting to evaluate how the patient's age could influence the judgment of the examining veterinarian for the early diagnosis of CHD. Taroni *et al.* [62] revealed a significant increase in the DI between 4 and 6 months and a significant decrease between 6 and 12 months, with DI values significantly lower at 12 months than at 4 months. In particular, dogs with a DI > 0.7 at 4 months showed a significant decrease at 6 and 12 months. Furthermore, also the results published in older studies described a similar but not significant trend in which the DI increases after 4 months until 6 to 8 months and then decreases at 12 months [33, 49] All these data underline how hip laxity can be influenced by the time of radiographic evaluation.

Until now, the LI has been considered a repeatable and reproducible parameter, useful for obtain an early diagnosis in young dogs [21, 41, 46, 55]. Because of our results, we cannot support the claim that the LI is a repeatable measurement for inexperienced operators and reproducible for all operators, but the mean difference was so small that we can continue to agree on its usefulness for early diagnosis in dogs, especially if evaluated in combination with other parameters. In fact, the LI is one factor considered in order to obtain the total score (TS), which represents the sum of all quantitative parameters evaluated during clinical and radiographic evaluations [AS + AR + (LI×10) + DARS] (AS, subluxation angle; AR reduction angle; DARS, dorsal acetabular rim slope) [55]. The TS, the combination of several parameters, provides a more reliably predictive value for hip dysplasia diagnosis [21, 55]. Nevertheless, in the literature there are doubts regarding its breed interpretation. In a recent study of Bertal *et al*. [54] the LI was compared with FCI grading revealing a moderate-to-good correlation between them: worse FCI grades corresponded to higher LI, but FCI A and FCI B grades (normal or almost normal hip joint) didn't exclude high LI values (min – max, 0.15 – 0.64 and 0.18 – 0.75, respectively). Dogs phenotypically normal based on the FCI grading had a wide range of joint laxity and hip joints of some breeds showed more laxity than others. The authors found a slight but significantly greater laxity in the Golden Retrievers compared to the Labrador Retrievers [54]. Our data also showed a higher laxity in Golden Retriever dogs and a lower laxity in Border Collie dogs of the same age, but these data were not correlated to the diagnosis of CHD and FCI score, so a statistical comparison of the results and any difference detected would not have provided further information. This observation, however, underlines the need for further investigation of LI breed-specific cut off value in order to guarantee an individual approach for each patient.

One limitation of the study is that the acquisition of the radiographs was taken from different radiological machines and literature reported that the image quality and the positioning could affect the assessment [34, 53]. Therefore, in order to overcome this limit, we performed a selection of acquired images and we included in the study only dogs with good image quality and an adequate positioning, in according to the guidelines [21]. In addition, the radiographic examination was also carried out by different performers simulating what happens in everyday practice. In order to ensure an excellent image quality and proper positioning and overcome the limits that this could have entailed, all performers, despite their different degree of experience, attended a theoretical-practical course that laid down guidelines for the radiographic image collection for this study. Bertal *et al.* previously demonstrated the technical repeatability and reproducibility of the VMBDD technique [13] and, using the PennHIP method, authors showed that the relationship between the applied force by operator on tibias and the degree of femoral head laxity was sigmoidal [63]. So, the force applied by different performers could be of varying magnitude, but the passive hip laxity obtained should not be influenced [13, 63]. However, despite this, this topic was not relevant for our purpose: to evaluate the reproducibility and repeatability of the LI. A further limitation was the absence of a comparison of the LIs with the FCI score evaluated in the adult subject, which did not allow us to provide interesting observations that would help clarify many doubts about the interpretation of the LI.

5. Conclusion

In conclusion, the LI measurement on radiographic images obtained with the VMBDD technique was a repeatable procedure if performed by examiners with moderate or high experience, but this could not be claimed for examiners with no or minimal experience. Notwithstanding this, the mean difference between the measurements performed during the three sessions by each observer was such that it could be negligible in clinical practice due to the low impact on the final diagnosis.

In addition, the LI appeared reproducible in our study only if performed by examiners with moderate or high experience. Nevertheless, even in this case, the mean differences between the measurements of the observers for each session could be negligible in clinical setting.

Therefore, the LI remained a useful measurement in combination with other parameters for early diagnosis of CHD in young dogs, but it is important to be aware that this parameter may vary depending on the operator performing the measurement and that this variability is reduced by the examiner's experience. In addition, in some individuals, delineating the acetabulum may be difficult and identify possible anatomical differences of acetabulum and the femoral head in different breeds could be interesting in order to be aware of anatomical reference points variations.

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