Assessment of the Immature Canine Coxo-Femoral Joint In Accordance with Graf's Technique

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ABSTRACT
Assessment of the acetabular morphology by ultrasonography is performed early in life in human neonates to screen for developmental dysplasia of the hip. The aim of this descriptive anatomic study was to determine whether ultrasound can be used to evaluate hip joint morphology in dogs. A preliminary cadaveric study was undertaken. An ultrasonographic examination was performed on the right coxofemoral joint of 10 canine cadavers, 4-12 weeks old. In Graf’s technique, anatomical parameters such as cartilaginous coverage of the hip, bony coverage of the hip and bony roof angle are evaluated. The technique was validated anatomically by sectioning the pelvis in the plane of the ultrasonic examination. The technique was validated anatomically by sectioning the pelvis in the plane of the ultrasound examination. Excellent correlation was found between the US images and the sectioned cadavers. Additional 72 hips (36 dogs, <8 weeks old) were examined using the technique validated in the first part of the study. The anatomical parameters described by Graf were determined from the scanned images. Three observers independently analysed the ultrasonograms on two separate occasions. Reliability was excellent for intra-reader measurements for two of the observers and moderate to high for inter-reader measurements of all the parameters, accept for the femoral head diameter. The technique described by Graf was easily adapted for use in dogs younger than 8 weeks old. High quality images can be obtained, which allow objective and subjective assessments of acetabular morphology. The ability of this technique to predict the development of hip dysplasia in dogs will need to be assessed in future studies.

Key words: Ultrasound; Hip Dysplasia; Dog.

INTRODUCTION
Canine hip dysplasia is the most common developmental orthopedic disease in dogs (1). Screening methods, which use the hip extended radiographic projection, have had relatively minor success in reducing the prevalence of this disease and despite intensive screening for decades; the prevalence of hip dysplasia is still as high as 40% in some breeds (2). Early diagnosis is of great interest especially in breeding colonies and working dogs. Hip extended and neutral-position radiography are the primary means used to identify young dogs with hip dysplasia and to eliminate these dogs from a breeding population. The Orthopedic Foundation of Animals limits the earliest hip screening in the US to dogs older than 2 years (2), however, the PennHIP procedure can be performed as early as 16 weeks with known accuracy (3-5). The age at which the diagnosis of hip dysplasia can be made using radiographic techniques is a limitation with the additional limitations of the need for a general anesthesia due to specific positioning requirements and exposure to ionizing radiation.

In infants, various techniques have been used to assess
hip morphology and stability at 4-6 weeks of age (6, 7).

Graf described a method that utilizes ultrasound to evaluate acetabular morphology and classify infant hips based on the depth and shape of the acetabulum (8). At this age, the femoral head is still cartilaginous and provides an acoustic window through which the acetabulum can be evaluated. The ultrasonographic image is used to assign hips to one of four categories. Each grade represents a particular acetabular morphology with the higher grades associated with increasing dysplasia (9). The overall specificity and sensitivity of ultrasonographic diagnosis of developmental dislocation of the hip in humans are reported to be 88.5% and 96.7% respectively (11).

Few studies have evaluated static and dynamic ultrasonographic assessment of the immature canine hip joint, those had a limited statistical power and varying degrees of success (12-16).

The aims of this study were to determine whether static ultrasonic images of the hip joint based on Graf’s technique, accurately represent the anatomy of the joint, to define the age window in which ultrasonographic evaluation of the hips can be performed reliably, and to evaluate the inter-observer and intra-observer variability of the technique.

MATERIALS AND METHODS

This study was designed as a descriptive anatomic study. No live animals were used for this study. Dogs included in this study were euthanized in line with population control regulations and donated for this study. Dogs were included in the study were evaluated by two of the authors (JM, AS) and were considered for inclusion if were 4-12 weeks old. Small breed dogs were excluded from the study, as well as dogs with any history of any pathology involving the hind limbs.

Initially, a pilot study was undertaken to establish a repeatable method for the acquisition and capture of diagnostic quality ultrasonographic images of the hip joint as described by Graf in five, 4-10 week old dogs. During the pilot study, the position of the probe relative to the hip joint was determined, and the position of the hind limb at the time of diagnostic quality image capture was measured using a goniometer. The technique determined in the pilot study was used throughout the study.

In the second phase of the study, ten, mixed breed, canine cadavers, weighing 5-6 kg and estimated to be between 4-12 weeks of age were used for the anatomic validation of the ultrasonographic technique and defining the timeframe in which ultrasonographic evaluation of the hips can be performed.

In the last part of the study, an additional 72 hip joints from 36 puppies less than 8 weeks of age were examined and the inter-observer and intra-observer variability of the technique was determined.

All canine cadavers were euthanized in line with population control regulations and donated for this study with permission of the institutional animal care use committee (# KSVM/VTH/32_2017).

Ultrasonographic technique

Ultrasonography was performed using a Mindray DC-8 ultrasound system (Shenzhen Mindray bio-medical Electronics Co., Ltd., Shenzen, China). The ultrasound technique used in this study is based on the technique described by Graf (9). Each cadaver was placed in lateral recumbency. The skin over the lateral aspect of the pelvis was clipped from the wing of the ilium to the trochanter major. Ultrasound coupling gel was applied to the shaved area (Figure 1). A linear array, 7 MHz probe, in B mode was used for all the ultrasonographic examinations. All ultrasonographic examinations were performed by an ultrasonographer experienced in the technique.

Anatomic validation of the ultrasonographic technique

Each of the 10 canine cadavers, used in this phase of the study, was placed on a separate wooden board in left lateral recumbency. The ultrasonographic examination of the hip joint was performed as described above. The plane of the ultrasound image was marked with two intramedullary pins as follows: immediately after the image of the hip joint was captured, and without altering the position of the ultrasound probe, an intramedullary pin was aligned parallel to the plane of the ultrasound image at the cranial end of the ultrasound probe, and advanced through the cadaver until it penetrated the wooden board. The same procedure was repeated with an intramedullary pin orientated parallel to the plane of the probe at the caudal end of the ultrasound probe.

The cadaver, fixed to the wooden board with the two intramedullary pins was then frozen at -30°C. Once frozen, the cadaver was cut with an industrial band saw in the plane defined by the intramedullary pins. The cut surface was im-
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Immediately cleaned of debris and photographed with a digital camera (Figure 2A). Care was taken to ensure that the plane of the cut surface of the specimen was parallel to that of the camera and that a constant camera to object distance was maintained. The digital photograph of the cut specimen and that of the ultrasonographic image (Figure 2B) were then compared.

Ultrasound image mensuration

Once the anatomic validation of the technique had been completed, both hip joints of additional 36 canine cadavers were examined, using the technique described above. All the examinations were performed by a single individual.

The images were measured as described by Graf (Figure 3A) using commercial image manipulation software (Fiji).
The baseline was placed on the image such that it was parallel to and superimposed on the lateral cortex of the ilium. The cartilage roofline was defined as a line extending from the lateral edge of the ilium to the center of the labrum. The cartilage roofline and the baseline define the "cartilage roof angle" (β) which characterizes the cartilaginous development of the acetabulum. The bony roofline was defined as a line extending from the medial to the lateral ilial edge. The bony roofline and the baseline define the bony roof angle (α) which characterizes the bony development of the acetabulum.

Two additional parameters, cartilaginous coverage and bony coverage, were calculated from each image. To calculate both of these parameters a circle was superimposed on the femoral head and the diameter of the circle measured. To calculate the cartilaginous coverage, the distance from the labrum to the medial aspect of the acetabulum was divided by the diameter of the femoral head. Similarly, the bony coverage (BC) was calculated by dividing the distance from the lateral to the medial ilial edge by the diameter of the femoral head. All measurements were performed twice, independently, by three of the authors (AS, JM, SY).

Once the measurements were completed, the hips were assigned to one of the categories according to Graf’s methodology where normal hips were defined as those having an α angle of ≥60°, and were classified as either type Ia or Ib depending on the age of the child. Hips with α angle ≤49° were defined as pathological, and were classified as types IIc, D, IIIa, IIIb, or IV (17, 18).

**Statistical analysis**

Continuous parameters were expressed as mean and standard deviation (SD). Intra-reader (within reader) and inter-reader (between readers) variability was assessed by calculating Cronbach’s alpha. This analysis was carried out for all joints and for all subgroups of joints (measured in three different positions). Data analysis was performed using statistical software (SPSS 17.0 for Windows, Chicago, IL, USA). P<0.05 was considered statistically significant. No power analysis was performed as this was an anatomical study.

**RESULTS**

The ideal image which contained the landmarks described by Graf were obtained when the hind limb was held in 10° adduction, 10° of internal rotation and 70° of flexion. The ultrasound probe was placed on the skin such that the long axis of the probe coincided with the center of the femoral head caudally, and bisected the wing of the ilium cranially. The hip joint was imaged in a lateral to medial direction. On the initial image, the curved line of acoustic reflection representing the femoral head and a similar line representing the ilium could be seen. An image acceptable for evaluation was one in which both the labrum and the medial wall of the...
acetabulum could be clearly seen and the line of the acoustic reflection of the ilium was horizontal (Figure 3A and 3B).

**Anatomic validation of the ultrasonographic technique**

The quality of US image was dependent on the extent of femoral head ossification. Dogs between 3-5 weeks of age had evidence of a secondary ossification center in the femoral head, but 3-5mm of cartilage was evident around the periphery of the femoral head. The secondary ossification center was larger and more organized in specimens of 6-8 week old dogs. Nonetheless, ultrasonographic images of the acetabulum could still be obtained. In specimens older than 8 weeks of age, ossification was almost complete, and as a result, identification of the anatomical landmarks was impossible.

There was an excellent correlation between the US images and the sectioned cadavers in cadavers 4-8 weeks old. The following structures were identified: labrum, ilial medial and lateral edges, ilial cortex and femoral head.

**Morphology of the hip joint**

The morphologic parameters as defined by Graf including the $\alpha$ and $\beta$ angles, cartilaginous coverage and bony coverage could be determined from all scanned US images. Mean cartilaginous coverage was 1.04±0.10, mean bony coverage was 0.82±0.09, mean $\alpha$ angle was 78.23°±3.09 and mean $\beta$ angle was 57.67°±5.36. All coxofemoral joints evaluated were non-pathologic according to Graf’s classification (between Grade I and Grade IIb).

**Inter- and intra-observer variation**

There was an excellent intra-observer agreement for two of the 3 observers (intra-observer variability >0.9) and moderate inter-observer variability for all parameters except the femoral head diameter (Table 1).

**DISCUSSION**

The results of this study demonstrate that the method described by Graf is applicable in dogs up to 8 weeks of age. The high quality images captured, correlated well with the anatomy of the hip joint, and allowed objective measurements of hip joint morphology, which had excellent intra-observer repeatability for most parameters and moderate inter-observer variability.

Canine hip dysplasia is one of the most common causes of hind limb lameness in dogs (19) and early diagnosis of this disease would facilitate both early surgical intervention and elimination of affected individuals from a breeding program. At present, static and dynamic radiographic techniques are used to identify affected dogs, however, despite being the most frequently used diagnostic tool, these have several disadvantages. Evaluating the hips between 12-24 months of age is associated with a minimum 30% error in diagnosis (20), and as a result the OFA requires evaluation of the hip when the dog’s age is above 24 months (21). Considering the limited ability to detect this pathology at an early age, radiographically, other methods should be explored. Ultrasound examination has the potential to detect hip dysplasia at a very young age. This will allow early intervention prior to the development of severe degenerative changes or even allowing preventative measures to attempt to prevent the development of the disease (e.g. symphysiodesis).

Our study utilizes an established static technique as a means of determining acetabular morphology. Few studies have evaluated the use of US for the screening of canine hip dysplasia (12, 14, 16, 22, 23). In these studies both static and dynamic ultrasonographic techniques have been used in young dogs with various degrees of success. A previous study evaluating this method concluded that there is no correlation between the ultrasonographic conformation of the hip and the final diagnosis of hip dysplasia based on radiographs obtained between 12-24 months of age (22). The above mentioned study (22) included a large number of dogs, however, only a very small number (14 joints) were avail-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Intra-observer variability</th>
<th>Inter-observer variability</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Reader 1</td>
<td>Reader 2</td>
</tr>
<tr>
<td>Femoral head diameter</td>
<td>0.87</td>
<td>0.93</td>
</tr>
<tr>
<td>Cartilage cover</td>
<td>0.85 (0.85**)</td>
<td>0.96 (0.94**)</td>
</tr>
<tr>
<td>Bony cover</td>
<td>0.86 (0.87**)</td>
<td>0.95 (0.93**)</td>
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<tr>
<td>Angle $\alpha$</td>
<td>0.59</td>
<td>0.96</td>
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<tr>
<td>Angle $\beta$</td>
<td>0.25</td>
<td>0.94</td>
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ICC: intra-class correlation coefficients using an absolute agreement definition, ** Mean intra-observer variability, absolute numbers.
able for follow up eventually developed hip dysplasia. When multivariate models were applied, the number of dogs with a final outcome of hip dysplasia highly influenced the statistical power (22). Thus, it was not surprising that this study was not able to present an accurate final predictive model and eroded confidence in the conclusions of the authors. Therefore, we believed that the assertion that Graf’s method was not correlated with the development of hip dysplasia as premature.

When using a radiograph to grade hip joints, there is no single parameter used for the final diagnosis, but rather the diagnosis is made using a combination thereof. Similarly, when assessing hip dysplasia using ultrasonography, a combination of parameters, rather than each one of these parameters independently, is expected to be more indicative of the presence or absence of the disease. It is therefore not surprising that previous studies have failed to show good correlation between single parameters (e.g., alfa angle) and the final grading (15).

In our study, three blinded investigators performed two measurements each, one week apart. There was excellent repeatability for two of the readers, as reflected by the low intra-observer variation. The inter-observer variability was lower, however, it compares favorably with previous studies reporting a wide range of inter-observer variabilities (24, 25). The various approaches to screening for hip dysplasia have conflicting data regarding their inter-observer agreement (20). All the observers were introduced to the technique at the time of the study, and though the inter-observer variability was acceptable, it is expected to improve with time and training.

The results of the present study demonstrate that the ultrasonographic evaluation of dogs up to 8 weeks of age is highly correlated with the hip anatomy, as was shown by the excellent agreement between the ultrasonographic findings and the cadaveric dissection. The technique is straightforward and can be easily applied to dogs of this age by a ultrasonographer trained in this technique.

In conclusion, the ultrasonographic technique described by Graf was easily adapted to dogs. In the hands of an experienced ultrasonographer, high quality images could be obtained and an objective and subjective assessments of acetabular morphology could be made. The cartilaginous femoral head provided an acoustic window through which the acetabulum may be imaged up to an age of 8 weeks. Acetabular morphology similar to that described by Graf in US examination of human acetabulae was recognized.

Further clinical studies are warranted to assess whether the grading system described by Graf can be used to predict the development of hip dysplasia in dogs.

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