Animal welfare/ethical statement: The present study was authorized by "Ethical Committee of the University of Pisa" (29914/18), according to the D.Lgs 26/14. Owners gave consent for their animals' inclusion in the study.

Giuseppe Conte<sup>c</sup>, Liri Ben David<sup>a</sup>, Simonetta Citi<sup>a</sup>

<sup>c</sup> Department of Agriculture, Food and Environment, University of Pisa, Pisa, Italy

<sup>a</sup> Department of Veterinary Sciences, Veterinary Teaching Hospital, University of Pisa, Pisa, Italy

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\* Corresponding author at: Irene Nocera, Veterinary Teaching Hospital, Department of Veterinary Sciences, University of Pisa, via Livornese snc, San Piero a Grado, 56122, Pisa, Italy.

E-mail address: irene.nocera@vet.unipi.it (I. Nocera).

## ABSTRACT

<sup>b</sup> Department of Equine and Small Animal Medicine, Veterinary Teaching Hospital, University of Helsinki, Helsinki, Finland

Ultrasonographic Appearance of Elbow Joints in a Population of

Irene Nocera<sup>a, \*</sup>, Benedetta Aliboni<sup>a</sup>, Micaela Sgorbini<sup>a</sup>, Luis Alfonso Gracia-Calvo<sup>b</sup>,

Ultrasound (US) is a well-established technique for investigating joint diseases in horses, complementary to radiography. Few studies have been performed on the ultrasonographic aspect of the elbow joint in horses and no reports are available on donkeys. The aim of this study is to describe the ultrasonographic appearance of the elbow joint in healthy donkeys. Descriptive cohort study included 34 elbow joints, which were evaluated in 17 donkeys. Inclusion criteria included no lameness or musculoskeletal diseases in the donkeys. The structures evaluated were the lateral and medial collateral ligaments, ulnaris lateralis proximal tendon, distal biceps brachii tendon, triceps brachii tendon, and the articular space. For each structure, one good-quality image was recorded. The structures were retrospectively assessed for echogenicity, fiber orientation, bone appearance, and shape. The prevalence of the visualized structures was calculated. Cohen  $\kappa$  coefficient was calculated for the repeatability (intraoperator agreement), the reproducibility (interoperator agreement), and the influence of the operator's experience in US examination. The US appearance of the structures was described. Statistical analysis showed scarce-tomoderate agreement concerning the repeatability and mostly scarce-to-good agreement concerning the reproducibility of the US examination; finally, low-to-discrete agreement concerning the operator's experience. Technical difficulties precluded an accurate description of the medial collateral ligament. The healthy animals included were limited. The US examination of the elbow joint in donkeys were similar to the features reported in horses. Individual experience partially influences the execution and the assessment of the US images.

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1. Introduction

The elbow is a large and complex joint because of its anatomical structure and position; and thus, can be difficult to assess properly [1]. For this reason, the evaluation and diagnosis of pathologies involving the elbow region may be delayed or not fully recognized [2].

The lameness associated with the elbow region is usually sudden in onset and generally moderate to severe [1-3]. Horses tend not to load weight on the lame limb, and it can thus be difficult to mobilize the limb. In the adult horses, lameness associated with the elbow is often related to direct trauma; in immature horses, stress fractures, osseous cyst-like lesions, and osteochondrosis are quite common [1-3].

The most common diagnostic tool used is the radiographic examination. This provides important information on the hard tissue

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structure; however, it is not as sensitive in the identification of soft tissue injuries [4]. Moreover, taking x-rays involves specific manipulations of the elbow region which if painful, might not be tolerated by the animal [1].

Ultrasonography is a fundamental and routine tool used in lameness examinations [4]. It provides fundamental information on the soft tissue structures of the elbow region, complementing the data obtained through radiography and nuclear scintigraphy [1,5]. It can also be helpful in detecting subtle focal changes in soft tissues related to this area [1].

Although the normal ultrasonographic appearance of the elbow joint in healthy horses has been described [1,6], there are few papers that report ultrasounds (USs) of the elbow area in the horses that are lame [5,7,8].

To the best of our knowledge, there are no studies on the normal appearance of healthy cubital joints in domestic donkeys. The aim of the present study was thus to ultrasonographically evaluate the elbow joint in healthy donkeys, and to describe and assess the appearance of anatomical areas liable to injury or degenerative disease. The study also assessed whether the technique was influenced by the operator's experience, both in terms of the execution of the US examination and the evaluation of the recorded images.

#### 2. Materials and Methods

#### 2.1. Animals

A cohort of 17 Amiata donkeys belonging to the Regional Stud Farm of Tuscany and housed at the Department of Veterinary Sciences of Pisa were enrolled in this study.

Four of 17 donkeys in the study were jacks, and 13 of 17 were jennies. The donkeys were aged between 7 and 19 years (median age: 9 years), the weight ranged between 235 and 324 kg (median 277 kg), and the body condition score ranged between 5 and 6/9 (median body condition score 5.5/9). All the donkeys were considered inactive and were used for reproductive purposes. Jennies were housed in collective paddocks 24 hours a day, whereas all the stallions were housed in individual  $6 \times 6 \text{ m}^2$  boxes with daily outside access. All donkeys were barefoot and underwent periodical hoof trimming every 50/60 days. They were fed with meadow hay *ad libitum* along with commercial equine feed concentrate in line with the National Research Council energy recommendations [9]. At the time of the study, no donkeys showed signs of musculoskeletal diseases and were considered healthy on the basis of a complete clinical examination.

## 2.2. US Technique

For all the donkeys enrolled, both forelimbs were scanned for a total of 34 joints. All the donkeys were held in a stock, and US was performed in the weight-bearing position, as previously reported [1,6]. Where necessary, US examination was performed under sedation (detomidine chloride, 10  $\mu$ g/kg, IV) to prevent a stressful situation for the patient.

The hair on the elbow region, delimited by the lateral and medial humeral condyles, the olecranon and the radial tuberosity, was clipped and shaved before the examination. Alcohol coupled with US gel was applied to provide appropriate contact. Ultrasonography was performed with a real-time B-mode with a portable US machine (MyLab30Gold, Esaote, Italy) using multifrequency linear and convex transducers. The following parameters were set: 7.5 MHz frequency, 82% gain, and 6 cm depth for the linear probe; 5 MHz frequency, 52% gain, and 6 cm depth for the convex probe.

The following anatomical structures were scanned and evaluated: lateral and medial collateral ligaments; *ulnaris lateralis*  proximal tendon, distal *biceps brachii* and *triceps brachii* tendons; *lacertus fibrosus*; and dorsal aspect of the humeroradial joint, in particular the articular cartilage of the humeral trochlea and the bone surface. As proposed by others for horses [6], three zones were defined for the examination of the collateral lateral ligament: zone A, origin of the ligament from the lateral humeral epicondyle; zone B, section of the ligament over the humeroradial joint; and zone C, insertion of the ligament at the proximal radius. In addition, for each zone, superficial and deep portions of the lateral collateral ligament were ultrasonographically detected and assessed [6].

All the anatomical structures were scanned using the linear probe except for the distal insertion of the *biceps brachii* tendon and the articular space, which were examined using the convex probe, as suggested in a previous study on the horse [1]. The US examination was performed in a craniodistal direction and each structure was evaluated in both longitudinal and transverse sections [6]. For each structure, at least one good-quality image was recorded. The evaluation was performed using dedicated software (MyLab Desk, Esaote, Genova, Italy).

An experienced veterinarian (IN) performed the US examination of 34 elbow joints. Then an even more experienced veterinarian (LAGC) evaluated the images offline, assessing the following features, in line with the literature [1,6]: the echogenicity (i.e., hyperechogenic, echogenic, hypoechogenic, and anechogenic), fiber orientation (i.e., homogeneous or heterogeneous), bone surface appearance (i.e., regular/irregular), and the shape (i.e., circular, oval, elliptic, and flat). In addition, for the *biceps brachii* tendon, the presence or absence of muscle fiber (i.e., yes/no) was evaluated.

### 2.3. Statistical Analysis

The prevalence for each structure visualized was calculated.

To verify the influence of the operator's experience in the evaluation of the US images, all the structures of the 34 elbow joints scanned were evaluated offline blindly by three veterinarians with different levels of experience (LBD: moderate; BA: fair; and IN: good) and compared by with the assessment performed by an optimal experienced veterinarian (LAGC). The agreement using Cohen  $\kappa$  coefficient calculation was carried out between veterinarians with moderate, fair, and good experience versus the veterinarian with optimal experience.

To evaluate the repeatability (intraoperator variability) of the examination, the three less-experienced veterinarians repeated the US on the same elbow joint on 3 consecutive days and the expert veterinarian evaluated the US images. The agreement using Cohen  $\kappa$  coefficient calculation was then carried out.

To evaluate the reproducibility (interoperator variability) of the examination, the US of the same elbow joint was performed by the three less-experienced veterinarians (LBD, BA, and IN), and the expert veterinarian (LAGC) did a blind evaluation of the US images offline. The agreement using Cohen  $\kappa$  coefficient calculation was calculated.

The kappa result was interpreted as follows: values  $\leq$  0 indicated no agreement, 0.01–0.40 slight, 0.41–0.60 moderate, 0.61–0.80 substantial, 0.81–1.00 optimal, NA: not appraisable. Statistical analysis was performed using JMP software (SAS Institute Inc., Cary, NC).

#### 3. Results

For all the animals, both elbow joints were scanned for a total of 34 US examinations. Sedation was only needed in 4 out of 17 (23.5%) animals.

In all the donkeys, all the lateral, cranial, and caudal elbow joint structures were easily visualized (34 of 34, 100%). These included

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laterally to the elbow, the lateral collateral ligament, and the *ulnaris lateralis* proximal tendon; cranially, the distal biceps tendon and the *lacertus fibrosus*; and caudally, the *triceps brachii* tendon. In addition, the lateral aspect of the articular space and the bone surface were also easily visualized in all the joints assessed (34 of 34, 100%). In contrast, the scanning procedure was difficult for the medial portion of the joint, and the medial collateral ligament was visualized in 15 of 34 of the elbows (44%).

The superficial portion in zones A and B of the lateral collateral ligament (Fig. 1) showed a regular and longitudinal fiber arrangement and a moderate echogenicity in the longitudinal and transverse views, respectively. However, the fibers were less regular and hypoechogenic in the deep portion. Consequently, zone C showed an echogenic and homogenous appearance, in both superficial and deep portions. Finally, in the transverse view, the shape was circular in zone A, and became elliptic and then thinner in zones B and C, respectively.

The *ulnaris lateralis* proximal tendon (Fig. 2) was visualized caudally to the lateral collateral ligament. In the longitudinal view, the tendon mainly showed a hyperechogenic and heterogeneous appearance. In the transverse view, the tendon was almost elliptic, with an echogenic and heterogeneous appearance.

The medial collateral ligament (Fig. 3) was visualized in 15 of 34 elbow joints (44%) and only the proximal insertion was visible. The echogenicity of the ligament ranged from hyper- to echogenic, with a hetero-to homogenous fiber alignment, in both transverse and longitudinal sections.

The *triceps brachii* tendon (Fig. 4) was visualized positioning the probe over the most caudal part of the elbow joint, where the tendon inserts into the palpable olecranon tuberosity of the ulna. In the longitudinal section, the appearance of the tendon ranged from echogenic to hypoechogenic, and showed a homogeneous fiber alignment; and the tendon had a progressively flattened shape. In the transverse view, the tendon appeared oval to elliptic in shape and presented an echogenic and homogeneous appearance.



**Fig. 1.** (A) Anatomical view, lateral aspect of the donkey elbow joint. Normal lateral collateral ligament (black arrow). (B) US image of the lateral collateral ligament, lon-gitudinal section, zones A (yellow line) and B (red line). (C) US image of the lateral collateral ligament, longitudinal section, zone C (green line). The left side of the images is proximal, the right side is distal. B-mode, linear probe 7.5 MHz. US, Ultrasound.

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**Fig. 2.** (A) Anatomical view, lateral aspect of the donkey elbow joint. Normal *ulnaris lateralis* tendon (black arrow). (B) US image of the *ulnaris lateralis* tendon, longitudinal section (within yellow lines). The left side of the images is proximal, the right side is distal. B-mode, linear probe 7.5 MHz. US, Ultrasound.

The *biceps brachii* tendon (Fig. 5) was localized on the craniomedial aspect of the elbow. In the longitudinal view, the tendon showed a largely hyperechogenic and heterogeneous fiber arrangement. The tendon was also slightly convex and flattened when it passed over the humeral trochlea. In the transverse view, the *biceps brachii* tendon showed an irregular shape and a hyperechogenic and heterogeneous appearance. Muscle fiber was detected in 16 of 34 (47%) joints.



**Fig. 3.** (A) Anatomical view, medial aspect of the donkey elbow joint. Normal medial collateral ligament (black arrow). (B) US image of the medial collateral ligament, longitudinal section (within yellow lines). The left side of the images is proximal, and the right side is distal. B-mode, linear probe 7.5 MHz. US, Ultrasound.



Fig. 4. (A) Anatomical view, caudal aspect of the donkey elbow joint. Normal *triceps brachii* tendon (black arrow). (B) US image of the *triceps brachii* tendon, longitudinal section (within yellow lines). The left side of the images is proximal, and the right side is distal. B-mode, linear probe 7.5 MHz. US, Ultrasound.

The *lacertus fibrosus* (Fig. 6) was identified cranially and laterally to the *biceps brachii* tendon, with a homogenous and hyper-echogenic fiber structure.

The articular surface of the humeral trochlea was visualized distally and slightly laterally to the *biceps brachii* tendon. The articular surface appeared regular and as an anechoic thin layer between hyperechoic lines of the humeral and radial bone surfaces. An irregular bone surface was detected in only 5 of 34 (14.7%) joints.

There was generally only a slight agreement in the evaluation of the images between the three veterinarians with different levels of experience compared with the expert veterinarian. Cohen  $\kappa$  test mostly showed slight-to-moderate agreement in the evaluation of both repeatability and reproducibility.



**Fig. 5.** (A) Anatomical view, medial aspect of the donkey elbow joint. Normal *biceps brachii* tendon (black arrow). (B) Anatomical view, cranial aspect of the donkey elbow joint. Normal *biceps brachii* tendon (black arrow). (C) US image of the *biceps brachii* tendon, longitudinal section (within yellow lines). The right side of the images is proximal, the left side is distal. B-mode, convex probe 5 MHz. US, Ultrasound.

#### 4. Discussion

Although there are studies that describe the US appearance of the elbow joint in healthy horses [1,6,10], there are few papers on donkeys. Chopin et al. [7] used US combined with radiography in the diagnosis of elbow pathologies. US was useful in the evaluation of the severity of soft tissue damage and early bone surface remodeling when associated with other diagnostic imaging techniques, such as radiography and nuclear scintigraphy [5,8]. An US of the elbow joint was also used to diagnose a growing lesion revealed by radiography in a 10-year-old gelding donkey [11].

To the best of our knowledge, this is the first report on the ultrasonographic evaluation of the elbow joint in a population of domestic donkeys.

US examinations were accepted by almost all the animals in this study and sedation was only needed in 4 out of 17 donkeys, in agreement with other studies performed on horses where sedation was usually not needed [1]. The need for sedation is mainly related to the animal's individual temperament, both for horses and donkeys [12].

US examinations of the lateral collateral ligament, the distal *biceps* and *triceps brachii* tendon, the *ulnaris lateralis* proximal tendon, and the articular cartilage of the humeral trochlea were straightforward, as also found for horses [1,10].

Assessment of the ultrasonographic characteristics of the lateral collateral ligament highlighted two different portions, both in zones A and B: a superficial portion with an echogenic and homogenous appearance, and a deep portion with a hypogenic and heterogenous appearance. The zone C US showed an echogenic and homogenous appearance in all the areas analyzed. These findings were similar to horses concerning zone C, whereas zones A and B mainly showed a heterogeneous appearance [1,6]. The lateral collateral ligament cross section was circular in zone A, becoming thinner and more elliptic in zone C, in line with the appearance in horses [1,6].

In our study, the echogenicity of the *ulnaris lateralis* proximal tendon ranged from hyper- to echogenic, in agreement with findings reported in horses [6,10]; however, in donkeys, a heterogenous appearance was shown, in contrast with a well-defined parallel fiber pattern found in horses [6]. This could be related to the hypoechoic fiber of the *ulnaris lateralis* muscle, which caudally surrounds the tendon and is not well differentiated in donkeys [6].



Fig. 6. (A) Anatomical view, cranial aspect of the donkey elbow joint. Normal *lacertus fibrosus* (black arrow). (B) US image of the *lacertus fibrosus*, longitudinal section (within yellow lines). The left side of the images is proximal, and the right side is distal. B-mode, linear probe 7.5 MHz. US, Ultrasound.

There were technical complications with the medial collateral ligament scans, possibly related to the anatomical location of the ligament and the muscular mass of the *pectoralis* muscles which hinders the visualization of this structure, as already reported for horses [1,6]. In line with the literature [1], access to the medial collateral ligament was difficult and required more expertise in the assessment. The inability to obtain an adequate visualization of the ligament, and thus the lack of an accurate description, is thus a limitation of the present study.

The *triceps brachii* tendon showed an echogenic and homogenous appearance and gradually flattened over the olecranon tuberosity, in agreement with findings for horses [1,6,10].

The *biceps brachii* tendon appeared hyperechogenic and heterogeneous, in accordance with the literature on horses [1,6,10]. In fact, for horses, the heterogeneous pattern was normal and was due to the presence of hypoechoic muscle fiber [1]. In our study, muscle fiber was detected in 16 of 34 of the tendons (47%).

The *lacertus fibrosus* showed a homogenous and hyperechogenic pattern. To the our knowledge, there is currently no description of the ultrasonographic appearance of *lacertus fibrosus* in the horse, thus it was not possible to assess any differences in appearance between donkeys and horses.

The articular surface of the humeral trochlea presented anechogenic cartilage and a regular bone surface, as with horses [1,6,10]. In the present study, 5 of 34 joints (14.7%) presented an irregular bone surface, but this was not associated with any clinical signs. The ultrasonographic evidence of an irregular bone surface with no clinical signs was also not considered as being relevant in horses [1].

The proximal insertion of the medial collateral ligament varied in appearance, ranging from hyper- to echogenic with a hetero- to homogenous fiber alignment. These findings are not in line with those found for horses, in which the ligament showed a linear and echogenic structure [1,6,10]. This might be related to the difficulty in scanning the medial aspect of the elbow joint, leading to an imperfect alignment between the probe and ligament.

Concerning the execution of the elbow joint US, the evaluation of the US images of the elbow structures seems to be influenced by the veterinarian's experience. Moreover, both intra- and interoperator variability need to be considered. In particular, the medial collateral ligament seems to be the most difficult structure to assess.

The number of donkeys included in the present study was limited, and a larger study group would provide more accurate data for the assessment of a normal ultrasonographic appearance of the elbow joint in the Amiata donkey breed.

## 5. Conclusions

To the best of our knowledge, this is the first report on the evaluation of the elbow joint in donkeys. Moreover, there appears to be no literature on the repeatability and reproducibility of the US execution or the influence of the operator's experience in the assessment of US images. Our results highlight that US examinations of the elbow joint need to be performed by veterinarians with a high level of experience to obtain good images and to interpret them accurately.

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#### Supplementary data

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