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SUMMARY OF Ph.D. THESIS

Ultrasonographic and Anatomopathological Study
of the Deep Digital Flexor Tendon Apparatus in the Equine Foot

SCIENTIFIC COORDINATORS,

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Outline of the Thesis

This study is divided in seven chapters organized in two main parts. The first three chapters form the Background bibliographic analysis and the last four ones are representing the Own research. The thesis has 255 pages, 114 figures, 49 tables and 209 references. Some of the research findings were reflected in the 5 articles and 6 oral communications.

The first chapter consists in a review of the descriptive and functional anatomy of DDFT apparatus. The second chapter approaches the morphology and molecular structure of tendons, ligaments and enthesis, as well as the pathophysiology of their injuries. The last chapter from the bibliographic analysis is referring to the ultrasound physics and instrumentation.

The own research is divided in four main chapters that present the epidemiology, ultrasonographic, anatomopathological, histopathological and magnetic resonance findings of DDFT, injuries in the distal limb at suprassesamoidean, sesamoidean and infrASSESamoidean level. The General conclusions and recommendations chapter is closing the manuscript.

My doctoral studies were carried out at University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania, University of Caen Basse-Normandie, France and CIRALE - Hippolia (Centre d’Imagerie et de Recherche sur les Affections Locomotrices Equines) Goustranville, France. During the Ph.D. program I benefited from a scholarship financed by European Social Fond according to Contract POSDRU/6/1.5/S/20 signed between the Romanian Minister of Work, Family and Social Protection and University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania.
OWN RESEARCH

CHAPTER 4

Retrospective study of 100 cases diagnosed with deep digital flexor tendinopathy in the distal limb: ultrasonographic technique and findings

The purpose of this study was to evaluate and to analyze the clinical interest, applicability and limits of diagnostic ultrasonography as an imaging method for diagnosing deep digital flexor apparatus injuries.

Materials and methods

Horse Population and Inclusion Criteria

Ten healthy horses, belonging to CIRALE\(^1\) - with no history of lameness and without any clinical sign like local swelling or joint distension were ultrasounded in order to obtain reference images of the equine deep digital flexor apparatus.

The retrospective study was performed on one hundred horses diagnosed with deep digital flexor distal tendinopathy at CIRALE using ultrasound examination. The cases were examined from January 16, 2009 to June 21, 2010. All horses included in the study had unilateral or bilateral forelimb lameness.

Imaging Examination Technique – the ultrasound exam

The ultrasonographic examination of the foot has been done with a non-portable machine Aloka Prosound Alpha \(^2\). Palmar and transcuneal approaches have been used. For each approach both longitudinal and transverse sections were realized. Convex and microconvex multifrequency transducers, with a frequency varying between 5.0 to 10.0 MHz have been used. The most often used frequency was 7.5 MHz. The distal part of DDFT was divided in three regions related to the distal sesamoid bone: supracesamoidean, sesamoidean and infracesamoidean. A lesion grading system from I to IV of ultrasonographic changes correlated with the chronic evolution of deep digital flexor tendinopathies has been established.

\(^1\) CIRALE - Hippolia (Centre d’Imagerie et de Recherche sur les Affections Locomotrices Equines) Goustranville, France

\(^2\) Aloka Co. Ltd., 6-22-1 Mure, Mitaka-shi, Tokyo, 181, Japan
Results

Ultrasonographic Anatomy of the Deep Digital Flexor Apparatus

The palmar ultrasonographic approach of the foot allowed us to examine the proximo-palmar aspect of the deep digital flexor tendon apparatus. The DDFT is bilobed with a heterogeneous structure. The podotrochlear bursa fluid is anechogenic and the membrane is thin and hypoechogenic. The distal digital annular ligament is represented by a hypoechoic line and it can’t be always visualized. The transcuneal approach allowed us to examine the distal aspect of the deep digital flexor apparatus.

Evaluation of Ultrasound Imaging as a Diagnostic Method for Identification of Deep Digital Flexor Apparatus Injuries

The main ultrasound findings of the deep digital flexor tendon were represented by changes in: size, echogenicity and architecture. The suprasesoamodean part of DDFT was most easily imaged by ultrasound exam. The most frequent modification of DDFT in the suprasesoamodean region consisted of thickening of one or both lobes (88%) and loss of their symmetry. Dorsal contour irregularities were present and more severe cases displayed dorsal hernias. Echogenicity was not particularly altered (increased or decreased) in our study group. The ultrasound imaging of the DDFT in the sesamoidean region is difficult. In the infrasesoamodean region 16 horses presented focal lesions and 21 horses presented multifocal lesions.

In 28 cases modifications in size and echogenicity of the distal digital annular ligament were also identified. In 53 cases podotrochlear bursitis was diagnosed. Thickening and proliferation of the synovial membrane were indicative of chronic podotrochlear bursitis.

Ultrasonographic Lesion Score of the Deep Digital Flexor Tendinopathies

Within the study group (n=100) that we analyzed the vast majority of lesions identified were grade 2 lesions. In this case the tendon presented a discreet lobe asymmetry accompanied by a mild heterogeneous echogenicity.
CHAPTER 5

Epidemiological study of deep digital flexor tendinopathy in the distal forelimb. Analysis of the study group (n=100 cases) compared to the general population.

The purpose of this study was to realize an epidemiological analysis of the population of horses diagnosed with distal deep digital flexor tendinopathies in the foot using ultrasonography. We were interested to establish the prevalence of this disease and to see if risk factors can be identified.

Materials and methods

Horse Population and Inclusion Criteria

The epidemiological study was performed on the same 100 horses that were used for the retrospective analysis in the previous chapter. In the same period 1380 horses were examined in the centre including the study group. The influence of breed, athletic (race or sport) activity, age and gender was assessed by comparing the injured horses with all the horses examined for lameness during the same time interval.

Statistical Analysis

The main objective of the statistical analysis was to evaluate the intensity of the factor-disease relation, which was estimated through the relative risk obtained by Odds Ratio value (ANOVA\(^3\)). The considered risk factors were: breed, age, sex and athletic (race or sport) activity. The statistical validity of the results, respectively Odds Ratio, was expressed according to the 95 % Confidence Interval (95% CI) and p value, calculated by Chi\(^2\) or Fischer Test (ANOVA). For all tests, a \(p\) value \(< 0.05\) was considered statistically significant. Pearson’s correlation coefficients were calculated to assess the associations between grade of the lesion and the variables taken in study.

Results

Breed Distribution in the Population Diagnosed with Deep Digital Flexor Tendinopathy

The major parts (64%) of the examined horses were Selle Français Warmbloods. The group of other Warmbloods was represented by 25 cases (25%). The following group was Anglo-Arabian horse and/or Arabian horse and French Trotter with 3 cases each (3%).

\(^3\) GraphPad InStat, 1992-2009, GraphPad Software Inc.
followed by the group of other breeds with 5 cases. No Thoroughbred was present in our study group.

Comparing to the general population examined at CIRALE, there was a very significant tendency ($p=0.0001$) for Selle Français horses to develop a DDF tendinopathy. They presented a 3.02 risk ($OR=3.02$) compared to the other breeds. In our study group French Trotters were less exposed to this condition, $OR=0.1056$, $p=0.0001$, and Thoroughbred horses were never diagnosed with it.

**Athletic (race or sport) Activity Distribution in the Population Diagnosed with Deep Digital Flexor Tendinopathy**

The majority of the horses included in this study were show jumpers (71%). The following category was represented by dressage horses (8%) and three day-event horses (5%). Trotters represented 3%. Five horses had other activities. No gallop racing horse was present in the study group.

It was an extremely significant tendency ($p=0.0001$) for show jumpers to have a high relative risk ($OR=3.3380$) to develop a distal tendinopathy of deep digital flexor tendon. According to statistical analysis, trotting was a protective factor for this type of pathology, the relative risk being very low, $OR=0.1100$, $p<0.0001$.

**Age Distribution in the Population Diagnosed with Deep Digital Flexor Tendinopathy**

The mean age of the horses diagnosed with deep digital flexor tendinopathy in the foot was 9.1 years. The age range for horses included in this study was between 3 years and 19 years. Standard deviation was 3 years and 0.9 months. The group 8 to 10 years old represents the majority of with 37%. In 88% of our cases horses were 6 years or older.

The most predisposed age category for a deep digital flexor tendinopathy were horses aged between 11 to 14 years ($OR=2.5890$, $p=0.0008$) followed by age category $>14$ years without statistical significance ($p=0.1034$). Horses younger than five years presented the lower relative risk for developing this pathologic entity ($OR=0.2914$, $p=0.0001$).

**Gender Distribution in the Population Diagnosed with Deep Digital Flexor Tendinopathy**

The population of our study was composed of 49 females, 40 geldings and 11 males. It is noticeable that there is a significant tendency in our study group for females to develop this pathologic entity compared to the general population ($p=0.0119$). They presented a 1.71 higher relative risk ($OR=1.71$) compared to geldings and males for DDF tendinopathy.
According to $p$ (0.0003) and OR values (OR=0.3417) males had the lowest risk to develop deep digital flexor tendinopathy. It is noticeable that the relative risk is increased for geldings, compared to males (OR=1.119), but this result is not statistically significant ($p=0.6737$).

**Association between the Lesion Grade and Breed, Athletic Activity, Age and Gender**

For all grade II and III lesions the correlation to horse breed was strong ($p \leq 0.001$). Irrespective of lesion grade, a direct correlation with the horses’ athletic activity was noticed ($r^2=0.9817-0.9971; p \leq 0.001$. For all IV degree lesions, the correlation with activity was not significant ($p=0.04$). Grade I, II and III lesions displayed a direct correlation with the animals’ age, ($r^2=0.6703-0.8686; p=0.02$ to 0.04). A correlation with negative trend was identified for grade IV lesions and age (95% CI: -0.50 to 0.96) without statistical significance ($p=0.28$). No significant correlation could be established between lesion grade and the animal’s sex ($p>0.05$).

**CHAPTER 6**

**Comparative Study of the Ultrasonographic, Anatomopathological and Histopathological Examination of Deep Digital Flexor Injuries on Isolated Limbs**

The purpose of this study was to do a detailed presentation of the anatomopathological and histopathological findings of the DDFT in the distal limb and to compare the sensitivity and the specificity of the ultrasound exam to anatomopathological and histopathological exams, in order to validate the use of ultrasonography in imaging lesions of distal part of DDFT.

**Materials and Methods**

This study was performed on 36 isolated thoracic limbs collected from 36 skeletally mature horses from Alba County (Romania) and Alençon (France). The DDFA was examined using ultrasound and gross post-mortem examinations followed by histopathology. For each region 4 longitudinal histological sections were examined giving a total of 16 sections per limb. Tissues were embedded in paraffin wax and sectioned at 5 µm. The Goldner’s Trichrome staining method was used.
Statistical Analysis

The statistical validity of the sensitivity and specificity of ultrasound exam was expressed according to the 95% Confidence Interval (95% CI) and p value calculated by Fischer’s Exact Test (ANOVA)\(^4\). For all tests, a p value ≤ 0.05. A Diagnostic Test Calculator\(^5\) was used to determine diagnostic test characteristics: the likelihood ratios, the pre-test probability (prevalence) and the post-test probability of disease. The accuracy of ultrasound exam was also calculated.

Results

Ultrasound Exam Findings

Among the 36 examined limbs, on ultrasonographic examination 27 limbs were diagnosed with DDF tendinopathy. Four different lesion types have been recognized in the distal portion of the DDFT: global lesions, lesions of the dorsal surface, parasagittal and oblique splits and insertional lesions including enthesopathy. The global lesions were the most frequent ones, 27 out of 36 examined tendons, followed by insertional lesions in 13 cases and lesions of the dorsal surface in 10 out of 27 cases. Parasagittal splits were seen only in 2 limbs.

Anatomopathological and Histopathological Exam Findings

The main lesions of DDFT which can be noticed via gross post-mortem examination were represented by: global lesions, dorsal surface fibrillations, parasagittal splits, adhesions, insertional lesions including enthesopathy or multifocal tendinitis.

The global lesions were the most frequent ones, 28 out of 36 examined tendons. The macroscopic aspect of the intratendinous lesion was characterized by: thick connective interfascicular septae and fibrosis indicated by color changes (bright white to grayish-white) and a general marble aspect of the cut surface. Histologically, they consisted of core necrosis, fibroplasia and fibrosis associated with thick interfascicular septa (endotendon).

Yellow coloration of the facies flexoria of DDFT of different intensities occurred in more than half of the examined tendons (18/36). It affected only the sesamoidean region.

\(^4\) GraphPad InStat, 1992-2009, GraphPad Software Inc.

\(^5\) Diagnostic test calculator (version 2006032401).Copyright (c) 2002-2007 by Alan Schwartz <alansz@uic.edu>. This calculator is Free Software, available under the Clarified Artistic License.
Lesions of the dorsal surface were represented by fibrillations, abrasions and erosions of the dorsal surface of DDFT and they were noticed in 11 limbs. Histopathologically they were represented by areas of dystrophic necrosis of the tendinous fibers, reduced cell number or complete loss of tenocytes, fibroplasia and hypertrophy of the epitenonium due to neovascularization with perivascular edema.

The dorsal parasagittal splits occurred in the sesamoidean region of the DDFT and extended proximodistally. In our set of cases 2 tendons presented parasagittal splits and they were superficial. Histologically, the fissure was also noticed as a split extending from the dorsal surface of the tendon. Collagen necrosis, fibroplasia and septal pallor were seen together with tenochondrocytes along the split and no evidence of inflammatory response.

Adhesions appeared as groups of fibers interposed between the dorsal surface of DDFT and the flexor surface of distal sesamoid bone. They occurred in 3 limbs and they were associated with partial thickness flexor surface fibrocartilage erosions of DSB and congestion and thickening of the synovial membrane of the podotrochlear bursa, signs of focal chronic inflammation.

Insertional lesions were present in 13 tendons out of 36 and consisted of surface irregularities, thickening or atrophy of the tendon, small core lesions, hemorrhage or adhesions between DDFT and distal impar sesamoidean ligament. Histologically, these findings were represented by fibroplasia of the epitenonium, loose connective tissue aspect with an increased matrix, collagenolysis and degenerative lesions of the collagen fibers from the epitenonium, increased number of neo-formation vessels with young endothelium and thickening of the endotendon.

**Sensitivity and Specificity of the Ultrasound Exam for Detection of Anatomopathological and Histopathological Lesions of Deep Digital Flexor Tendon at Macroscopic Examination**

Our work hypothesis were confirmed, the ultrasonography had a very high overall sensitivity (96%, \( p < 0.0001 \) for the anatomopathological exam and 83.87%, \( p=0.0094 \) for the histopathological exam). The method was less specific (respectively 88.89% for the anatomopathological exam and 80.00% for the histopathological exam).

The ultrasonography of DDFT in the sesamoidean region was more difficult than proximal or distal to this segment due to the oblique orientation of the fibers and the anatomical particularities of this specific region. In this part the tendon has a hypoechoegenic
aspect and it is difficult to objectively identify a pathological variety of echogenity. In this region the ultrasound exam had the lowest specificity (38.10% for gross post-mortem exam and 35.71 for histopathological exam) with an increased number of false negatives, but the results were not statistical significant ($p=0.0514$ and $p=0.3884$).

In the infrasesamoidean region of DDFT the sensitivity of ultrasound (78.57% and 58.33%) was lower than specificity (86.36% and 91.67%) and these results were statistically very significant ($p=0.0002$ and $p=0.005$).

**CHAPTER 7**

*Comparative study regarding the contribution of low-field standing magnetic resonance imaging and ultrasonographic imaging in examining the deep digital flexor tendon injuries in the distal limb*

- The main objective of this chapter was to qualitatively compare the contribution of low-field standing magnetic resonance imaging and ultrasonographic imaging in examining deep digital flexor tendon (DDFT) injuries in the distal limb in horses with unilateral or bilateral forelimb lameness.

**Materials and Methods**

This study was performed on 28 horses with unilateral or bilateral forelimb lameness, with pain associated to deep digital flexor tendon injuries. All the horses were examined using ultrasonography and MRI at CIRALE between January 2010 and September 2011. A definitive diagnosis of DDFT apparatus was established after the magnetic resonance exam was performed. A total number of 50 front limbs were examined by both methods.

**Imaging Techniques**

*Radiologic Exam*

Digital radiologic examination was performed with an Indico 100 Rad X-Ray generator. An output of 70 Kv and 8 mAs was used. The lateromedial, weight-bearing, dorsopalmar, weight-bearing, 60° dorsoproximal-palmarodistal and palmaroproximal-palmarodistal (skyline) radiographic views were obtained.

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6 Communications & Power Industries Canada Inc. *communications & medical products division* 45 River Drive / Georgetown, Ontario / Canada L7G 2J4.
Ultrasound Exam

The technique of the ultrasound exam of DDFA was described in the previous chapters.

Magnetic Resonance Imaging Exam

All feet were imaged with a low magnetic field strength (0.27 Tesla) imaging unit. Transverse MR images were obtained using 3-dimensional (3D) T1 weighted gradient echo (GRE), 3D T1 weighted gradient echo (GRE) HR (perpendicular to the navicular bone), T2 FSE and STIR. Frontal images were obtained using 3D high resolution (HR) T2 weighted gradient echo (GRE) sequence. Sagittal images were obtained using short tau inversion recovery (STIR) fast spin echo (FSE) sequence and 3D T1 weighted gradient echo (GRE).

Statistical Analysis

The statistical validity of the sensitivity and specificity of ultrasound exam was expressed according to the 95% Confidence Interval (95% CI) and p value calculated by Fischer’s Exact Test (ANOVA). For all tests, a p value < 0.05 was considered significant. A Diagnostic Test Calculator was used to determine diagnostic test characteristics: the likelihood ratios, the pre-test probability (prevalence) and the post-test probability of disease. The accuracy of ultrasound exam was also calculated.

Results

Radiographic Findings

On the 28 horses, the radiographic exam of the foot was performed in 44 limbs out of 50 scanned with both ultrasound and magnetic resonance. Standard radiographic views were obtained. No significant radiological abnormality was detected in 19 limbs out of 44 (43.18%).

Ultrasound Exam Findings

Lesions of the DDFT were identified by ultrasound exam in 31 out of 50 scanned limbs. The ultrasonographic findings consisted of changes of size, echogenicity and architecture. Different lesion types have been recognized in the distal portion of the DDFT:

7 Equine Limb MRI Scanner (Eq2), Hallmarq Veterinary Imaging Ltd., Guildford, Surrey, UK
8 GraphPad InStat, 1992-2009, GraphPad Software Inc.
9 Diagnostic test calculator (version 2006032401).Copyright (c) 2002-2007 by Alan Schwartz <alansz@uic.edu>. This calculator is Free Software, available under the Clarified Artistic License.
global lesions, lesions of the dorsal surface, sagittal plane and oblique splits and insertional lesions including enthesopathy.

Global lesions were the most frequent ones, 28 out of 50 examined tendons. Most of them were identified in the suprasesamoidean region (20/28) and in the infrasesamoidean region (16/28). Dorsal surface irregularities were seen in 8 limbs and insertional lesions in 5 limbs.

*Magnetic Resonance Exam Findings*

Lesions of the DDFT were identified in 35 out of 50 scanned limbs. In most of the limbs (27/35) lesions within DDFT were mostly represented by signal alterations. Six limbs presented a high intensity signal in T1 and T2. In 5 limbs the signal was heterogeneous.

We have divided the DDFT injuries in global lesions and specific lesions. Global lesions were characterized by thickening of the tendon. The specific lesions were: dorsal lesions, core lesions, adhesions with the surrounding anatomical formations and insertion lesions.

Thickening of DDFT was encountered in 22/35 limbs. In 24 limbs out of 35 dorsal lesions were identified. Core lesions were seen in 3 cases and they were characterized by increased signal intensity in all image sequences. In all cases the medial lobe of DDFT was injured and the core lesion was associated with the thickening of the affected lobe. The parasagittal plane splits were seen in 6 cases. The proximodistal length of parasagittal split varied between 8 to 80 mm. Adhesions between the DDFT and neighboring anatomical formations were identified in 10 limbs. Insertional lesions were seen in 5 limbs. All these lesions were identified by ultrasound exam as well. They consisted of thickening of DDFT and signal changes.

*Sensitivity and Specificity of the Ultrasound Exam for Detection of Deep Digital Flexor Tendon Lesions Identified with MRI*

In the suprasesamoidean region the ultrasound exam had the highest sensitivity, 79.17% (95% CI: 57.84-92.87) and the highest specificity, 96.15% (95% CI: 80.38-99.90), extremely significant statistically (p<0.0001), with the ultrasound relating best to the MRI findings.

In the sesamoidean region the ultrasound exam had the lowest sensitivity, 21.43% (95% CI: 4.6 - 50.80) and a very good specificity, 94.44% (95% CI: 81.35 - 99.32), but the results were not statistical significant (p=0.1262).
As for the lesions detected using ultrasonography in the infrasesamoidean segment of the tendon, the sensitivity was 72.22% (95% CI: 46.55-90.30) and the specificity was 78.13% (95% CI: 60.02-90.74), being extremely significant (p=0.0008).

GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. Global epidemiological analysis revealed that the risk factors were represented by middle age or older horses (more than 7 years), females, Warmbloods which were used for show jumping. The protective factors were represented by young age (0 to 3 year old), males, Thoroughbreds and French trotters which were used for racing.

2. Most of the ultrasonographic findings were reconfirmed by macroscopic, microscopic and magnetic resonance exams. Histopathological findings support the degenerative etiopathogenesis of distal DDFT lesions.

3. The high sensitivity and specificity of ultrasonography in the suprasesamoidean and infrasesamoidean region validate its use for these segments of the tendon. The major disadvantage of the ultrasound exam is its low capacity to image the sesamoidean and parasagittal parts of DDFT.

4. The main advantages of this technique include low cost, short examination time, applicability in the field, good patient tolerance and capacity to recheck injuries during the horse follow up and rehabilitation.

5. In this survey histopathological examination of deep digital flexor tendon injuries revealed various vascular disorders. A practical recommendation would be to use the colour Doppler mode when examining the DDFT in the distal limb.

6. In the clinical assessment of this apparatus, a complete examination of soft tissues has to be complemented with the investigation of surrounding osseous formations: distal sesamoid bone and distal phalanx. In order to achieve this, ultrasonography is routinely applied as a complementary investigating method to radiology.

7. The MR exam is recommended to complement the ultrasound exam when the precise cause of distal limb lameness could not have been precisely located by other imaging techniques.