



TECHNICAL UNIVERSITY OF LISBON

Faculty of Veterinary Medicine

**Acquired flexural deformation of the distal interphalangeal joint in foals**

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ADVISOR

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CO-ADVISOR

Doctor Maria Luisa Mendes Jorge

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*“A butterfly flaps its wings in one part of the world and on the other side a hurricane happens.”*

Edward Lorenz

## THANKS

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## **TITLE: acquired flexural deformation of the distal interphalangeal joint in foals**

### **ABSTRACT**

Since 2008, the high prevalence of acquired flexural limb deformities front was observed in the Lusitano stud farm. This work aims to evaluate this problem by reporting the results from tissue alterations in the affected animals as well as environmental conditions and management changes, which could have led to this observation.

A total of eleven affected animals were studied. In these, a complete physical and orthopedic examination were performed specifically the determination of the angle between the dorsal hoof wall and the floor. Radiographic examination, CT imaging, determination of the thickness of the cortical bone of the third metacarpian and histopathology of some tissues collected in biopsy and necropsy were done in a subset of affected foals. All the animals had been supplemented with balanced commercial diet for equine. To investigate a possible genetic cause, two foals from distinct bloodlines were brought to the stud. These also developed the deformities after 6 months.

Two of the affected foals were placed in a pasture away from the initial one and two others were admitted at the College of Veterinary Medicine in Lisbon. In those animals, except for one that had to be euthanized for humane reasons, an improvement was observed on their condition, with partial recovery of the deformity.

Histopathology was performed from (i) the tendon obtained by surgical desmotomy in one foal, (ii) tendon biopsies were performed in three foals and (iii) from the tissue of one foal during necropsy.

Histologically the most significant alterations were the dissociation of myofibrils of the smooth muscle. This was predominantly seen in the small intestine but also in the walls of small capillary vessels, including those of the tendon vasculature.

The flexural deformities have a complex and multifactorial etiopathogeny. They occur due to uncoupling of the longitudinal development of the bone and its adjacent soft tissues, but also from shortening of the tendon-muscle unit in response to pain.

In the case series presented here, there was no obvious cause for the development of this problem, therefore we hypothesized of this condition, especially those introduced in recent years.

**KEY WORDS:** Acquired flexural deformity; distal interphalangeal joint; environmental conditions; foals.

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## **LIST OF ABBREVIATIONS AND ACRONYMS**

DDO - Development Orthopedic Disease

FD (FD) - flexural deformation

AFD (FDA) - acquired flexural deformation

FDDIJ (FAID) - flexural deformation of the distal interphalangeal joint

AFDDIJ (FDAAID) - acquired flexural deformation of the distal interphalangeal joint

CVM (FMV) - College of Veterinary Medicine

ALTDFD (LATFDP) - accessory ligament tendon deep digital flexor

ECM (MEC) - extracellular matrix

TAC - CT Scanner

WT (TE) - wind turbine

SFDT - Superficial digital flexor tendon

DFDP - deep digital flexor tendon



**Anex I**

**ICIST**



INSTITUTO  
SUPERIOR  
TÉCNICO

**MEASUREMENT OF MECHANICAL VIBRATIONS INDUCED  
BY WIND TOWER IN VILA SECA, TORRES VEDRAS**

Study for the College of Veterinary Medicine, Technical University of Lisbon

July 2012

**ICIST Report**

**EP No. 15/12**

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## 1. INTRODUCTION

This report summarizes the studies measuring mechanical vibrations in farm livestock, now identified simply by exploration, existing in Vila Seca, Torres Vedras. These studies were conducted by ICIST / IST (Institute of Structural Engineering, Territory and Construction, IST, Technical University of Lisbon) at the request of the College of Veterinary Medicine, also at the Technical University of Lisbon.

The operation in which the records were collected from mechanical vibrations devotes special attention to raising horses for bullfighting. The main objective of the studies is to characterize the mechanical vibrations induced in different locations of the holding for the operation of existing wind towers in their vicinity, in order to analyze the possible effects of the vibrations on the horses.

## 2. EXPERIMENTAL CAMPAIGN OF HARVEST RECORDS OF MECHANICAL VIBRATION

### 2.1. General

This section describes the methodology adopted, including the description of the collecting sites and equipment used, as well as identification registers.

### 2.2. Experimental Procedure

The methodology adopted was the collection of records triaxial vibration at different sites within the holding well as in some locations outside of it, closer to or away from the wind tower.

The instruments were installed to digitally record vibration on the ground, with varying durations, close to 1 minute.

The collection of the records was performed on two separate occasions: March 22, 2012 and June 25, 2012.

In the first data collection we were able to verify that in some sites there were potential abnormalities in the methodology - wind effects (measurement devices were not protected), and movement resulting from humans and animal proximity, in

general, in conjunction with the low level of existing environmental vibrations, made us realize that a second data collection was necessary.

In this second data collection measures were taken to assure the quality of the records in a way that they referred only to the effects of environmental vibration.

### 2.3. Areas of Measurement

Altogether the two occasions mentioned above we were able to collect records in six groups of sites, highlighted in Figure 1 and identified in the following list:

A - Next to a shed near the towers (A1, A2 and A3, depending on distance, decreasing for wind towers, making A3 located next to the fence nearest holding one of the towers, (39°07'26"N; 09°08'51"W);

B - Inside the barn nearest the towers (only the first data collection);

C - Inside the barn farthest away from the towers (only the first data collection);

D - Outside in an area away from the towers (plateau), (39°07'31"N; 09°09'01"W);

F - outside in northern boundary of the urban area of the small town of Folgorosa (39°08'00"N; 09°09'13"W; not represented in figure 1) only in the second data collection.

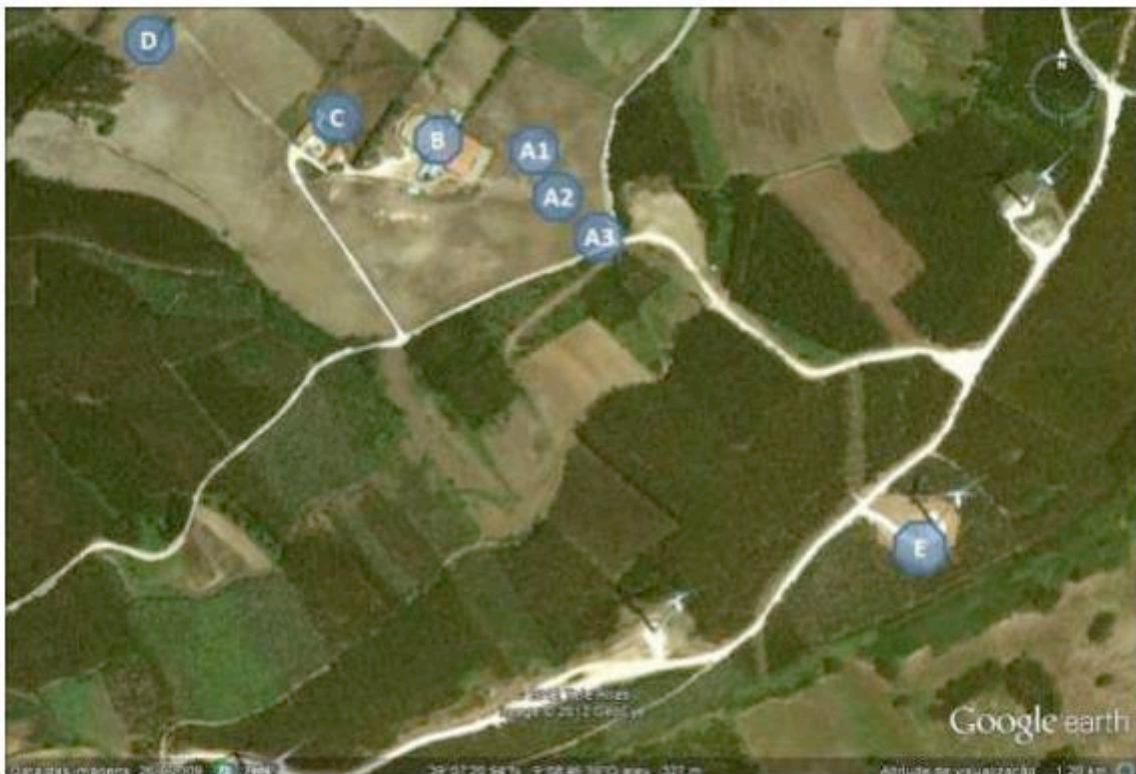


Figure 1: Plant with the location of the sampling collection areas (source: GoogleEarth).

As an indication, the distance between the nearest location (A3) of the zone of wind towers and wind tower nearest (E) is approximately 350m.

The distance between sites A1 and A3 is close to 100m and the distance of the farthest site in Figure 1 (D) and the nearest wind tower (E) is approximately 800m. The site (F) is located outside the farm in a remote area about 1750 meters from the towers.

## 2.4. Equipment

Vibration collections were made with the use of digital recording units triaxial accelerations Kinometrics brand, model ETNA (with internal sensor Episensor), similar to that shown in Figure 2.

On the first day of data collections three separate units were used while the second day is used only one unit.

The most relevant features of the unit record vibrations used are briefly indicated in the following list:

- Episensor triaxial sensor with the following specifications:
  - the dynamic range exceeding 135 dB
  - the sensor bandwidth from DC to 200 Hz
  - the linearity of  $<1000 \mu\text{g} / \text{g}^2$
  - hysteresis  $<0.1\%$  fullscale
  - the cross-sensitivity of less than 1% (including misalignment)
  - fullscale selectable by the hardware between 0.25g to 4g
- Drive signal conditioning, digitization and registration with the following specifications:
  - the storage of records in internal PCMCIA card
  - the conditioning and filtering analog signal, including through filtering low-band to cut 80% of the Nyquist frequency and 120 dB attenuation
  - analogical digital conversion performed with 18-bit resolution
  - storage of records in internal PCMCIA card

The unit used was configured with a full scale of 1g, which corresponds to a resolution in acceleration, after scanning,  $0.0008 \text{ cm} / \text{s}^2$ .



Figure 2: Triaxial unit of accelerations registration.

## 2.5. Identification of registers

As stated above, it was verified that records collected in the first data collection had some spurious effects, such as the wind and human and animal disturbance, so it was decided to conduct a second data collection. For that reason we mentioned in this document only the records collected in the second collection of data with the exception of those collected at the site (F). The identification of records is shown in Table 1.

Table 1 - Identification of registers (2nd collection)

Area	Records	Notes
A1	CX001	Tower 1 - Stopped Tower 2 - 47 spins/min Tower 3 - 33 spins/min
	CX002	
	CX003	
	CX004	
	CX005	
	CX006	
	CY001	
	CY002	
	CY003	
	CY004	
	CY005	
	CY006	
A3	CZ001	
	CZ002	
	CZ003	
	CZ004	
	CZ005	
	CZ006	
D	DA001	
	DA002	
	DA003	
	DA004	
	DA005	
	DA006	
F	DB001	
	DB002	
	DB003	
	DB004	
	DB005	
	DB006	
	DB007	
	DB008	

In all records held first and second collecting data acquisition rate was 250 Hz (250 SPS).

The wind speed during the second data collection was, according to information from the Institute of Meteorology, approximately 27km / h.

As the frequency of the movement of wind turbine blades, in both collections was variable (tower to tower and, in a given tower during the observation period) was between 9 and 15 total rotations per minute.

Among the measurements taken in the first collection, records CO001 and CO005, collected on the basis of the nearest wind tower (local E), in this case without disturbances from other sources.

### **3. ANALYSIS OF RECORDS**

#### **3.1. General**

The analysis of the records was conducted to correlate the intensity of ambient vibrations to the collection site, thus seeking to infer the extent to which environmental vibrations are affected by the operation of the wind towers.

In addition, we proceeded to a summary analysis of the frequency distribution, identifying the predominant frequencies.

#### **3.2. Intensity of the vibrations**

The vibration intensity can be inferred from the traces (acceleration versus time) of the components of the records.

We present below (Figures 3 and 12) the traces of some individual, grouped by direct order of the distance to the nearest wind towers.

We present only the image records corresponding to the horizontal components (X and Y), since the vertical component (Z) are of lesser amplitude. Indeed, we are able to see various registrations that the maximum values of the vertical component of the measured accelerations are, in general, equal to approximately 1/3 in some cases about 1/2 of the maximum values of the horizontal components. Although not shown, the values recorded in the first collection of measurements at sites B and C (inner two stables) are comparable with the values recorded at sites A1 to A3.



Area E (base of the nearest tower)

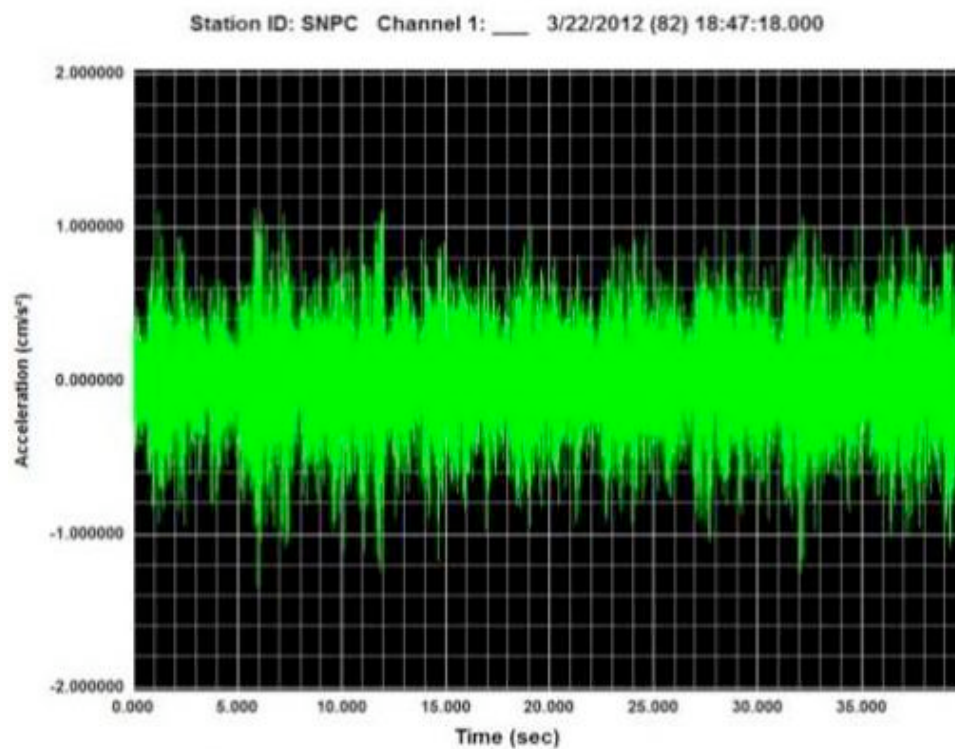


Figure 3: Trace of component X, diametrical registration CO002 (site E).

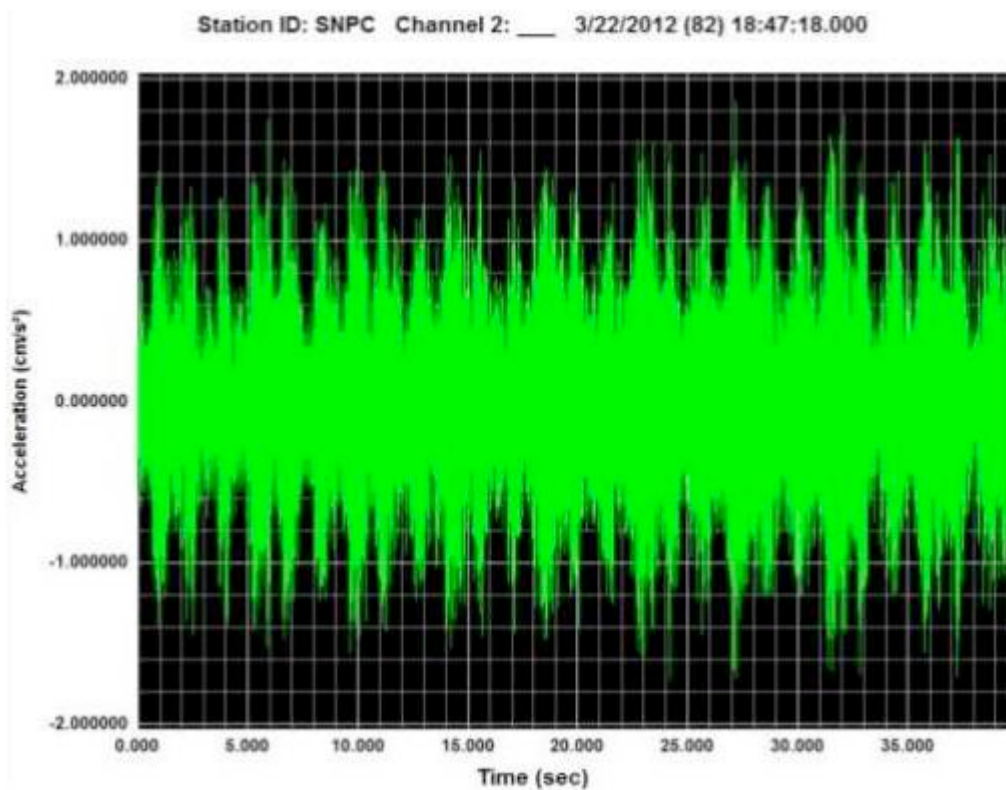


Figure 4: Tracing the Y component, radial, registration CO002 (site E).  
Local A3 (pasture, about 350m from the nearest tower)

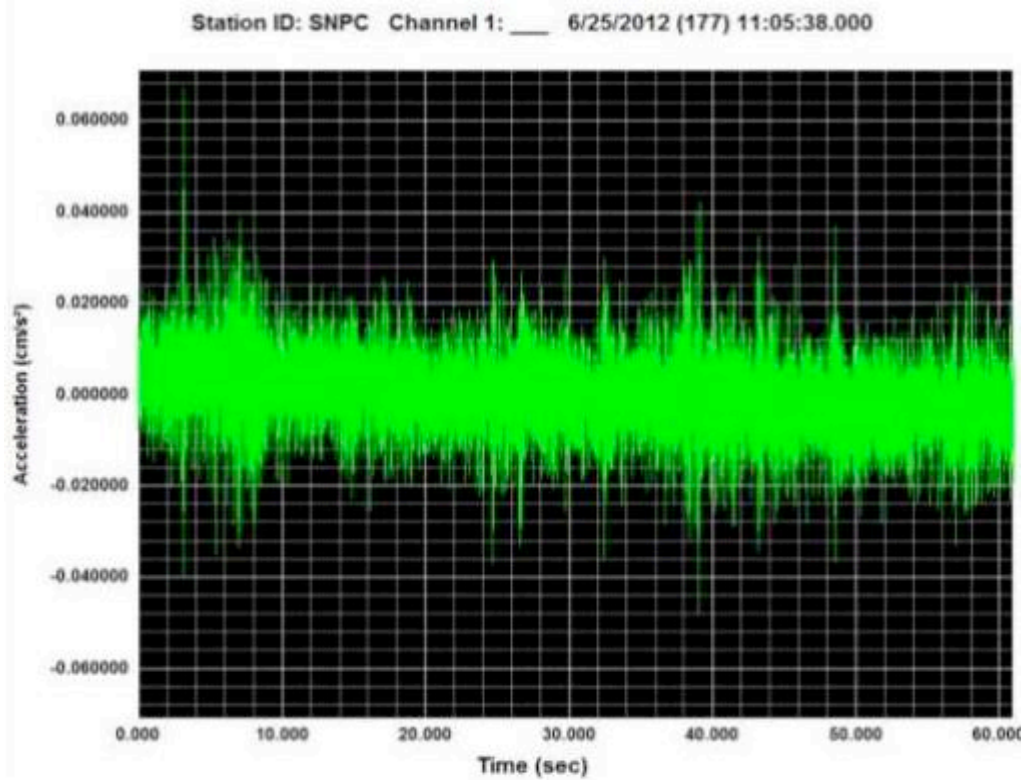


Figure 5: Trace of component X, diametrical registration CZ005 (site A3).

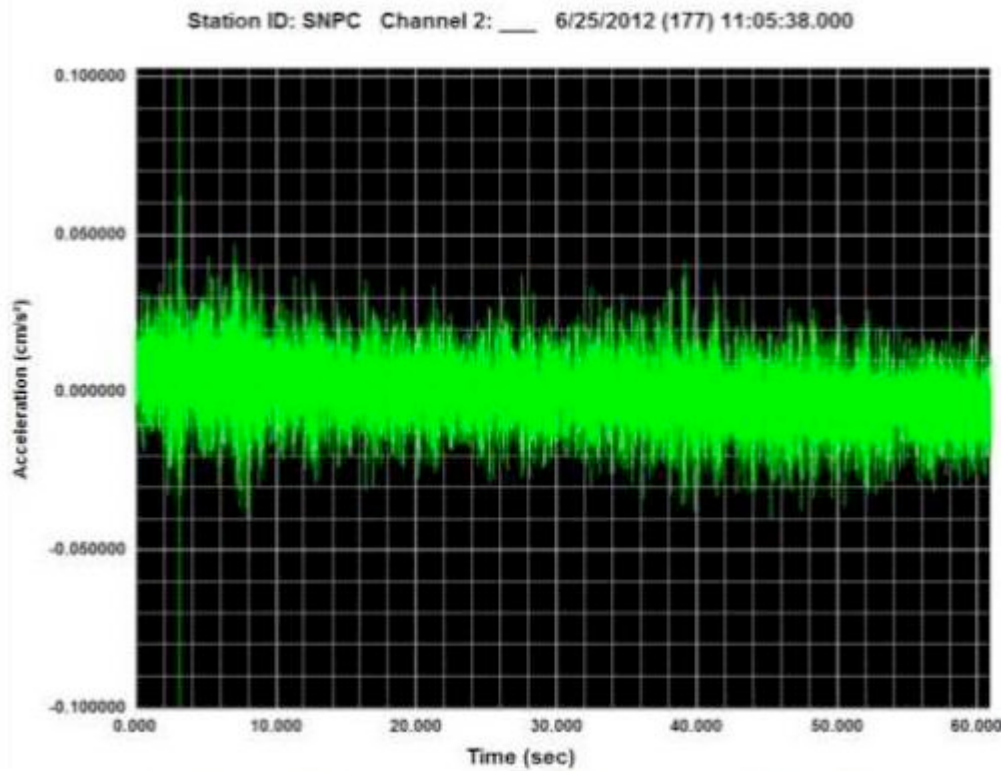


Figure 6: Tracing the Y component, radial, registration CZ005 (site A3).

Local A1 (pasture, about 450m from the nearest tower)

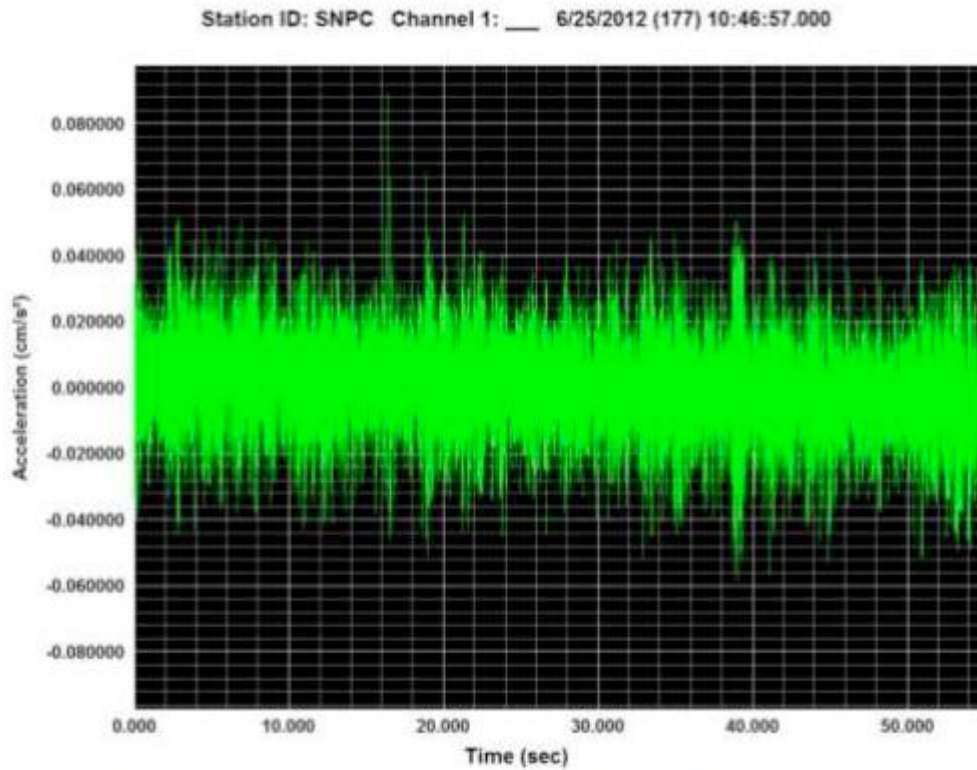


Figure 7: Trace of component X, diametrical registration CY005 (site A1).

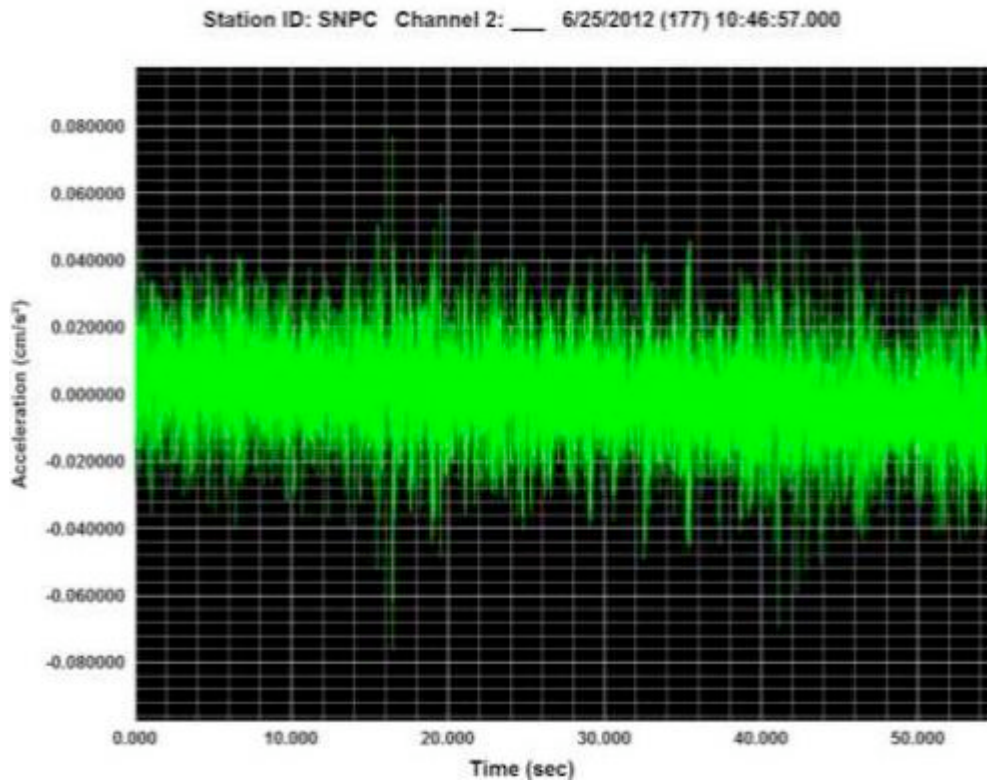


Figure 8: Tracing the Y component, radial, registration CY005 (site A1).



Local D (pasture, about 800m from the nearest tower)

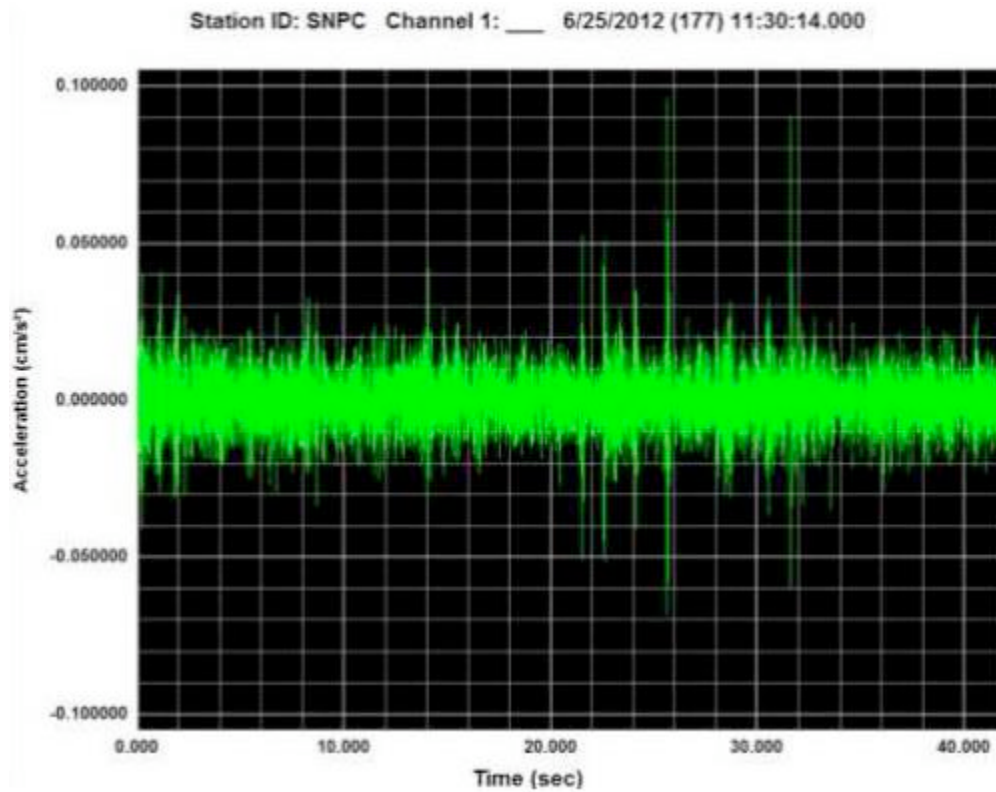


Figure 9: Trace of component X, diametrical, registration DA006 (site D).

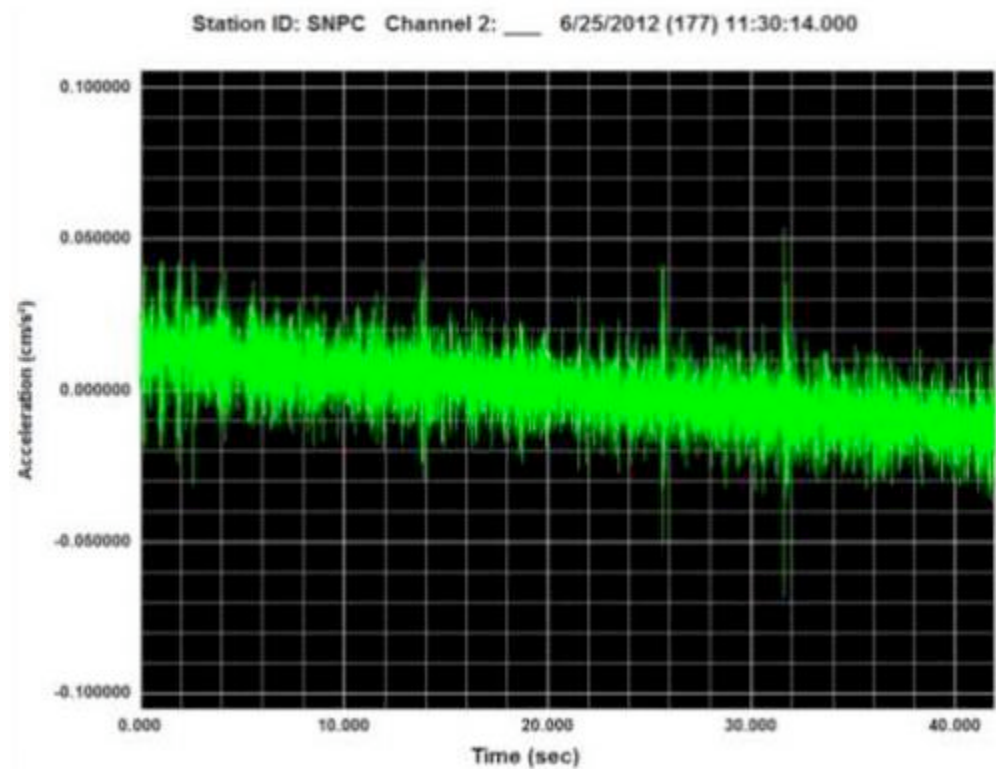


Figure 10: Trace of the Y component, radial, registration DA006 (site D).

Local F (farthest from the wind towers, off-farm)

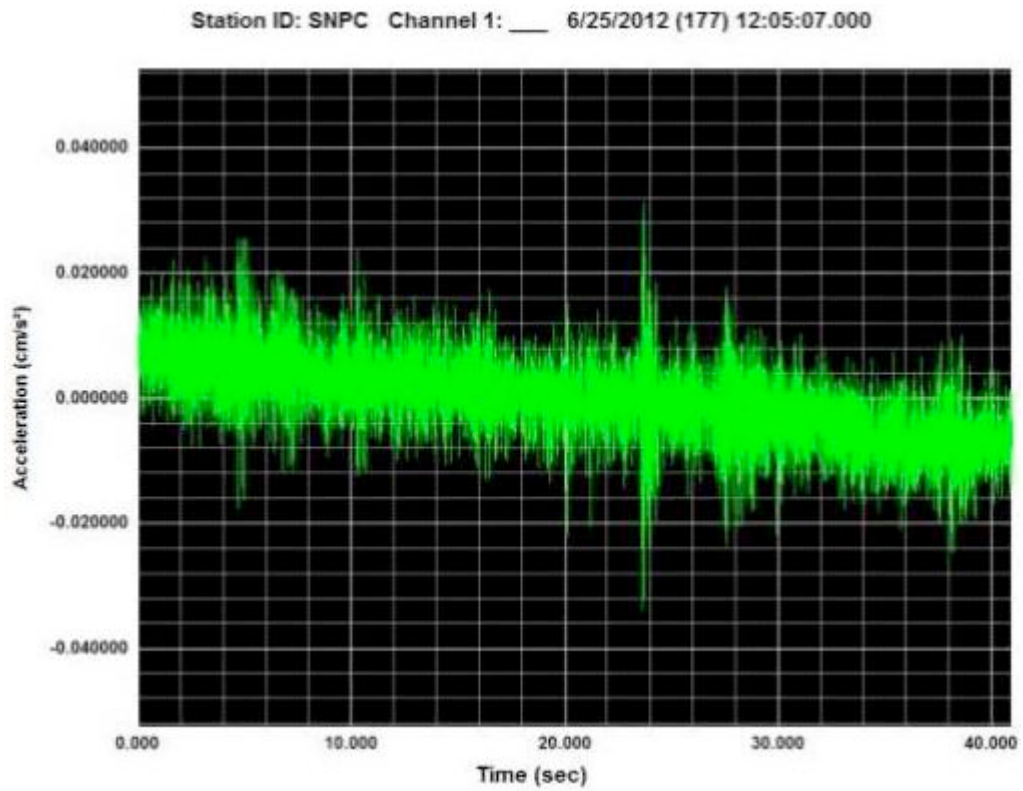


Figure 11: Trace of component X, diametrical registration DB005 (site F).

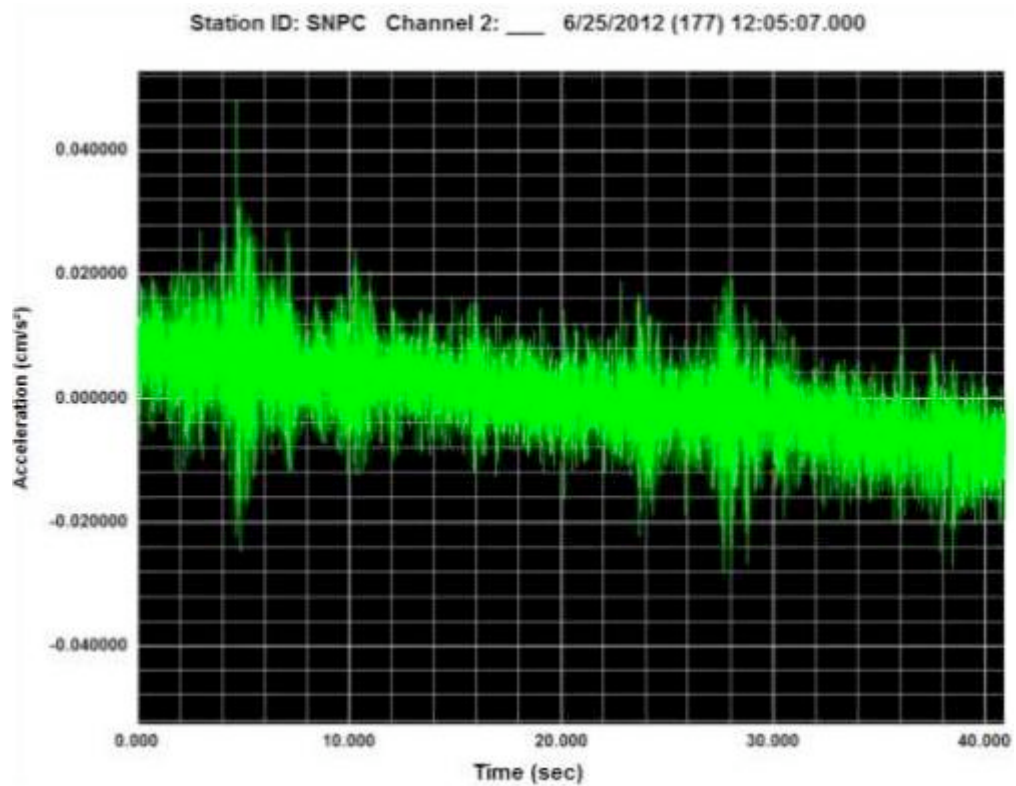


Figure 12: Trace of the Y component, radial, registration DB005 (site F).

From the observation of the previous figures (Figures 3 to 12), especially those relating to records of lower intensity (Figures 10 and 12) we can see a slight drift of readings (values are not always centered on zero acceleration), resulting from low intensity and, as will manifests a component of very low frequency (with a period of the order of magnitude of the length of the registers).

This does not preclude that conclusions can be drawn regarding the intensity of the vibrations at different locations and hence the attenuation of this intensity with distance from the wind towers.

In a way and considering all the records collected in different locations, we can see in Table 2 the maximum acceleration recorded (again adopts an order corresponding to the distance to the area of wind towers). We can see a range of variation of the maximum values (peak values).

Table 2: Maximum values of acceleration (radial and diametral)

Area	Diametral	Radial
E - base of the towers	0,9 to 1,1cm/s <sup>2</sup>	1,5 to 1,9 cm/s <sup>2</sup>
A3 - pasture, closest to the towers	0,03 to 0,04 cm/s <sup>2</sup>	0,03 to 0,04 cm/s <sup>2</sup>
A1 - pasture, less closest to the towers	0,04 to 0,05 cm/s <sup>2</sup>	0,04 to 0,05 cm/s <sup>2</sup>
D - pasture, far away from the towers	0,02 to 0,03 cm/s <sup>2</sup>	0,02 to 0,03 cm/s <sup>2</sup>
F - outside the farm, away from the towers	0,015 to 0,02 cm/s <sup>2</sup>	0,015 to 0,02 cm/s <sup>2</sup>

From the observation of Table 2 we can see that in terms of maximum values, the directional effect (difference between the diametral and radial components) is just clear to the wind tower.

### 3.3. Analysis of frequencies

Having concluded that the intensity of the vibrations within the range is reduced, the frequency analysis of the contents of registers was naturally shortened.

Notwithstanding that fact, we are able to see in the following figures the traces of the functions of the power spectral density (acceleration) of the records collected from the towers (location E) and a representative of the local conditions on the farm (site A1).

Local E (base of the tower closest)

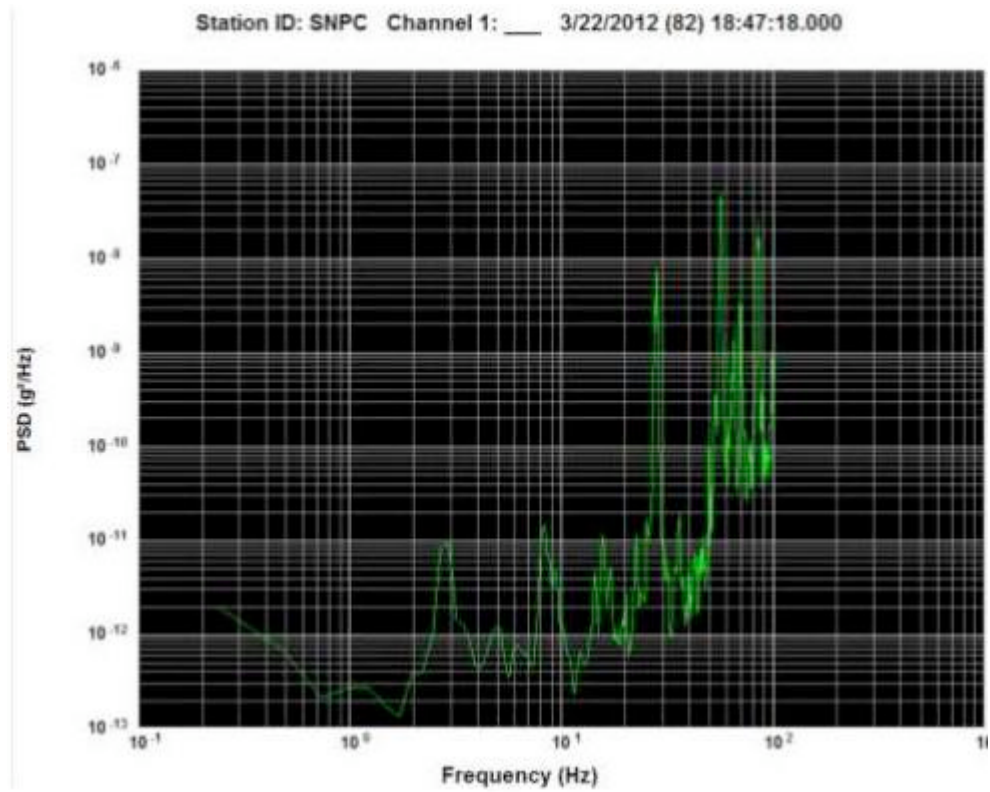


Figure 13: Frequency distribution of component X, a diametrical CO002 registration (location E).

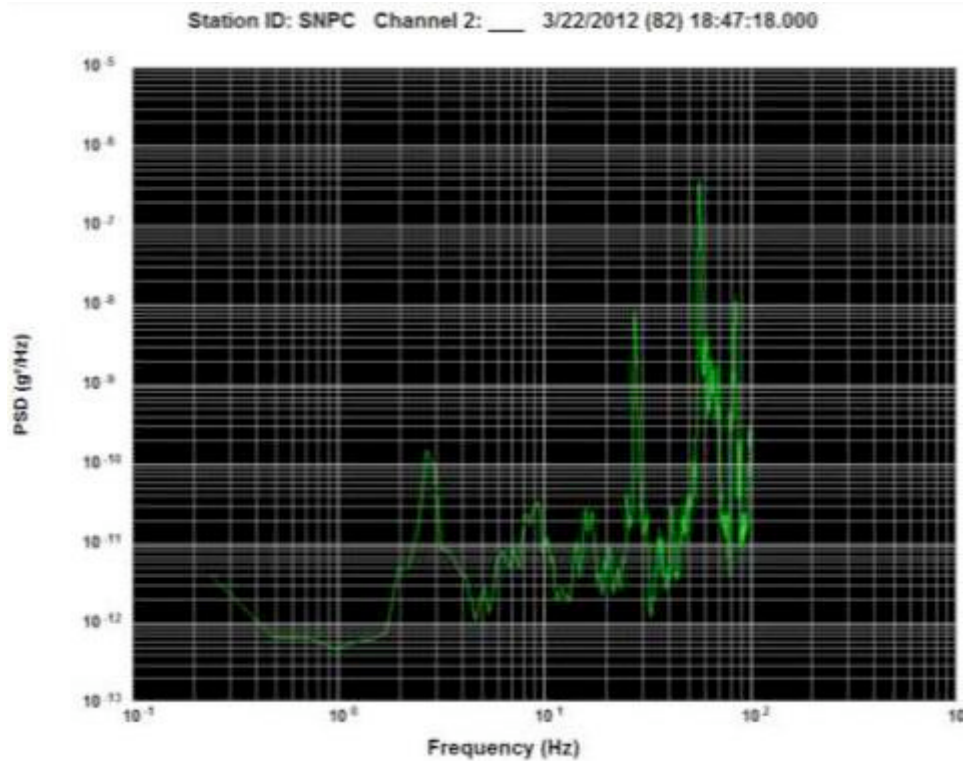


Figure 14: Distribution of the frequency component Y, radial registration CO002 (spot E).

Local A1 (pasture, about 450m from the nearest tower)

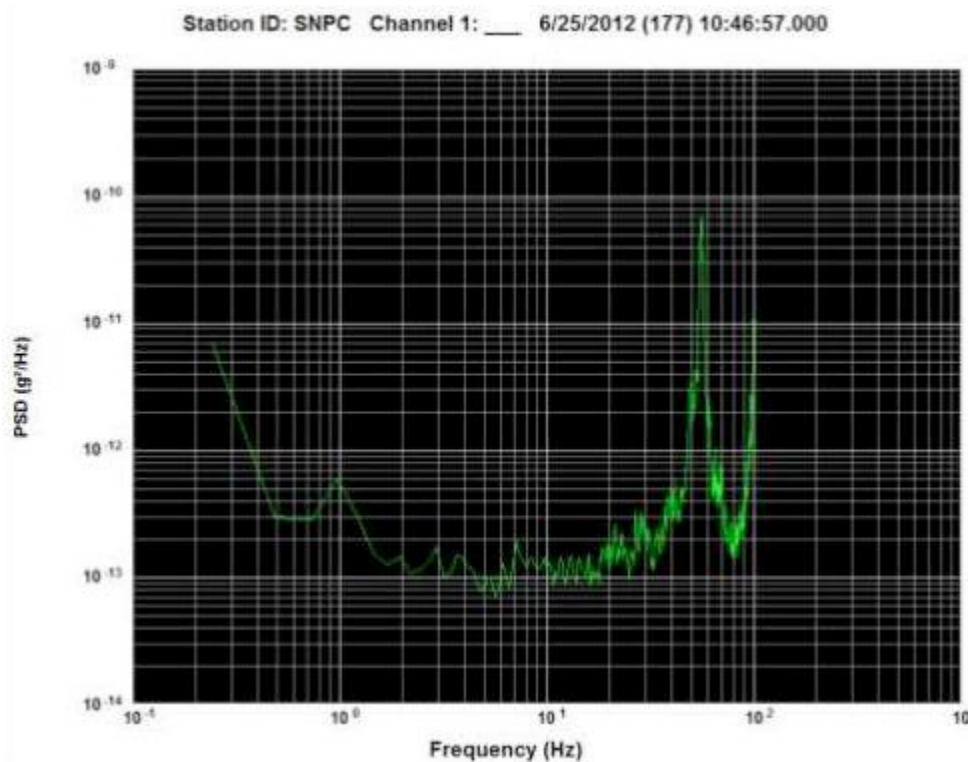


Figure 15: Frequency distribution of component X, a diametrical registration CY005 (local A1).

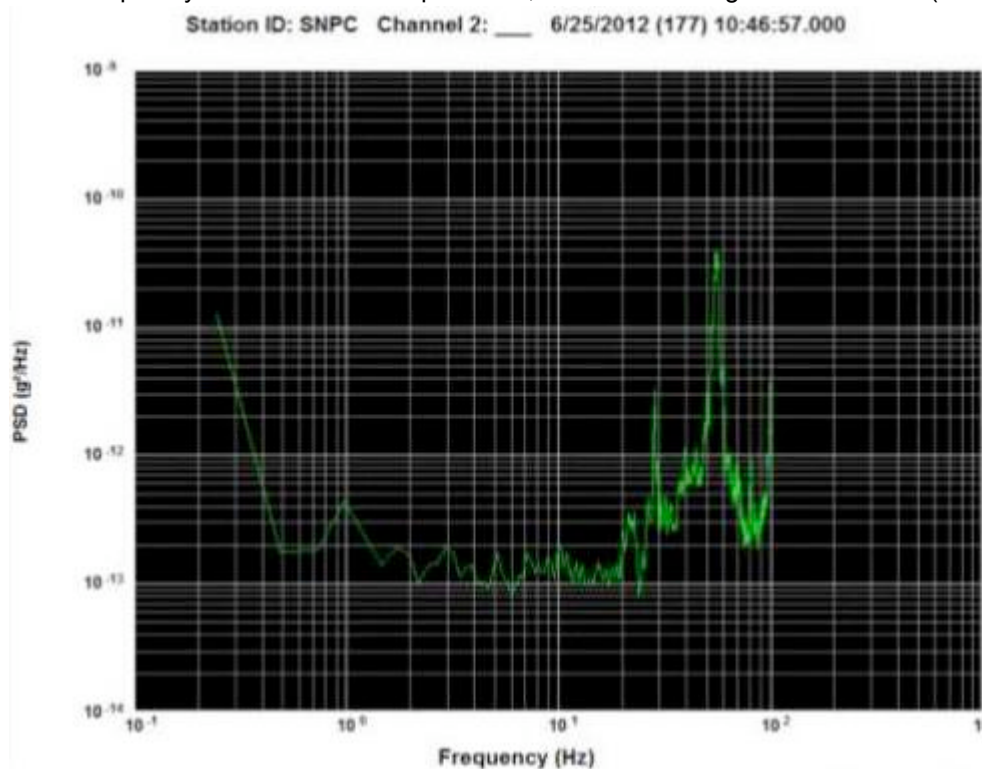


Figure 16: Distribution of the frequency component Y, radial registration CY005 (local A1).



From observing the preceding figures we can perceive the following conclusions:

- The vibrations induced by the operation of the wind towers (movement of the blades and the remaining existing mechanical equipment) have a frequency content concentrated in bands with medium frequency 3 Hz, 8 Hz, 15 Hz, 28 Hz, and especially around 55 Hz. We can see clearly that some of the bands may vary with the speed of movement of the blades (which in turn is correlated with wind speed).
- The vibrations within the existing operating in the frequency band concentrate, lower, at 30 Hz and above (again), close to 60 Hz. Possibly concentration around 30 Hz manifest especially in radial components (which involves transmitting waves compression of the soil).

#### **4. CONCLUSIONS**

The comparative analysis of previous results seem to point to the fact that the vibration induced by the operation of wind towers to attenuate very rapidly with distance from the source (comparing records obtained in E with those from A1 to A3).

Within the perimeter of the holding this attenuation is already quite low, such that the zone closest to the wind tower there is a direct correlation with distance and unambiguous the same towers (comparing logs obtained in A1, A2 and A3). The attenuation with distance is in a much more mitigated to the holding areas and areas farthest away from the holding considerably longer (comparing logs obtained in A1, A2 and A3 with those obtained in D and F also). It should be noted that there has been no analysis of the influence of wind speed on induced vibrations, since this intensity was comparable in the two campaigns of recording. The analysis performed in the frequency domain suggest a strong attenuation of the movements induced with lower frequencies, below 30 Hz, while to persist, though much attenuated ranges of frequency 30 Hz and near 50 Hz

Lisbon, July 6 2012

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President of ICIST

## **CHAPTER I – INTRODUCTION**

In a Lusitano Stud Farm, near Lisbon, municipality of Torres Vedras, (hereinafter described as Stud), from 2008, was observed, the development of flexural deformities acquired in the equine forelimb. In all foals born after that date, there was a marked hyperflexion of the distal interphalangeal joint, occurring in the same sagittal plane (Figure 1). In some cases, the lesion was observed bilaterally, with a degree of alteration of the angle flexural variable. The Stud has existed since 2000 and prior to 2008, this symptom has not been observed in any horse, and there was no change in the diet (quality and quantity), the conditions of housing and exercise regime to which the horses were be submitted. The pedigree of this horse stud also remained the same genetic lines from the top of it and some animals were introduced from different sources, both paternal and maternal that in the course of some months also exhibited the same sagittal deviation of the distal forelimbs, like other horses affected. The appearance of these lesions coincided with the installation of wind turbines (TE) 2 mega-watts that were implemented in the said land adjacent to the stud, which is the only environmental change that has occurred.

The degree of hyperflexion of the distal interphalangeal joint, was observed as variable among foals. However, some have lost the ability to support almost the entire sole of the hoof on the ground, supporting only the clip, undermining its locomotion very significantly and, concomitantly, their well-being and quality of life. Associated with the onset of flexural deformation also appeared several behavioral changes in horses, passing these over time to sleep during the day and providing longer periods decubitus.

The aim of this study was to evaluate the tissue damage present in affected animals, looking to estimate how far the stable environmental conditions may have interfered with the occurrence of acquired flexural deformation observed.

**Figure 1.** Flexural deformation of the distal interphalangeal joint of a stud in foals affected.



**Figure 2.** Aerial photographs showing the stud farm and wind turbines.



**Figure 3.** Grassland showing one of the stud breeding mares and a wind turbine near.

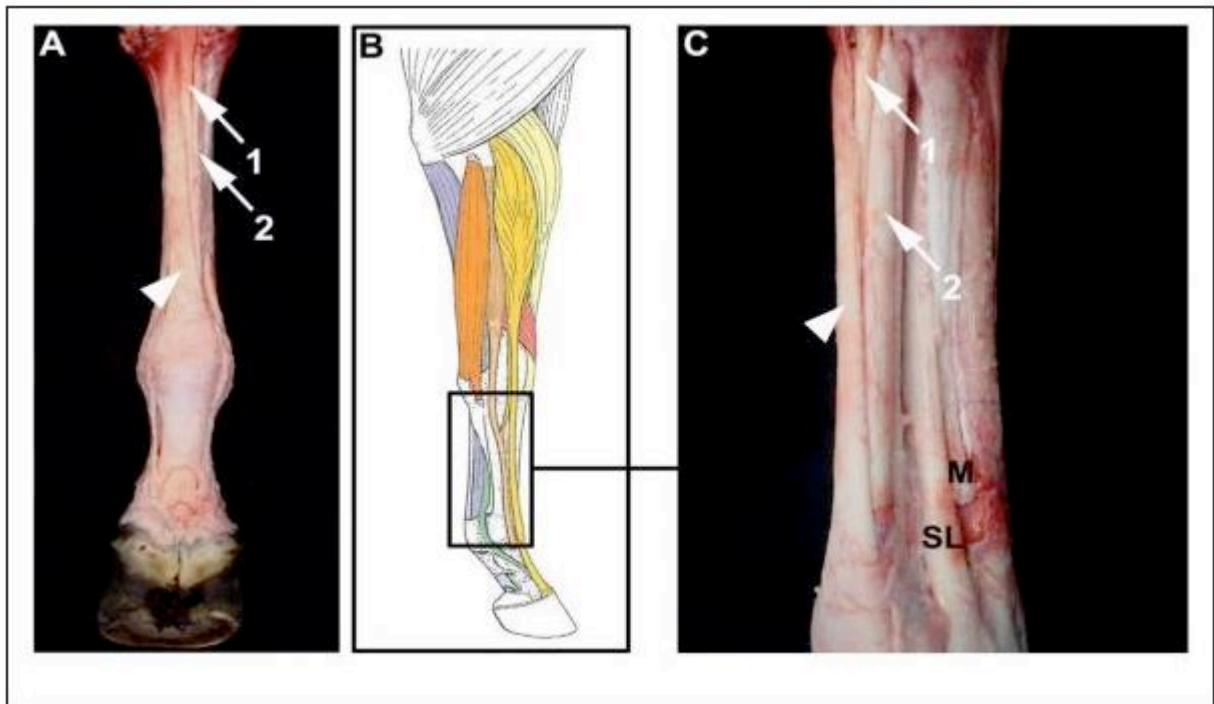


### 1.1. Flexural deformation (FD) in foals

Flexural deformations (FD) members represent a problem with plexus that is discussed frequently in the literature of veterinary medicine for horses. In this type of FD there is a deviation in the sagittal plane of the limb, which can be expressed by a hyperextension or hyperflexion of an articulation (Auer, 2006). The hyperflexion is colloquially called contraction of tendons, the tendons although not in fact contracted or altered; only are shorter on the associated bone structure. This condition is verified with greater frequency in forelimbs expression usually bilateral, though one of the members being more affected than the other, in most cases (Barr, 2002).

The FD presented at birth defects are classified as those that emerge later are designated for granted. These latter are considered as part of the development set of orthopedic diseases (DDO), in which also includes the angular deformations of the members, osteochondrosis, and epiphysitis cervical vertebral malformations (Bramlage, 1987).

**Figure 4. A distal equine forelimb. A.** Palmar aspect. **B.** Picture schematic side view (Adaptada de Anatomia de los Animales Domesticos; Konig HE, HG Liebich, 2001). **C.** Lateral aspect. **Spear Head.** Deep digital flexor tendon. **2.** Accessory ligament of the deep digital flexor tendon. **M.** Metacarpal IV. **SL.** Suspensory ligament of the fetlock.



### **1.1.1 Pathogenesis of acquired flexural deformities**

Etiopathogenesis the FD acquired is complex and multifactorial, and several theories have been proposed for its occurrence. The two most prevalent are, firstly, the gap between tendon and bone growth and, secondly, shortening of the muscle-tendon unit in response to a painful stimulus (Barr, 2002). As mentioned, the rapid bone growth may be one of the causes responsible for FD, it leads to a mismatch between the growth of bone in relation to the adjacent soft tissue structures tendons, ligaments and muscle.

The rate of bone growth is genetically determined, but also impacted by nutrition and mechanical forces applied to the bone. Excessive feeding of foals can occur when the mare has excessive lactation or there is an excessive supplement of concentrated feed. Changes in growth can also occur when food intake increases very suddenly, especially after a period of food shortage. When a flexural deformation occurs in foal at foot, the energy level of this diet should be reduced and, if necessary, weaning should be carried out and the mare's rations decreased. The balance of minerals present in the foal's diet, should be evaluated much like the mare, especially the relation between calcium and phosphorus, since several studies have indicated the involvement of imbalance of said minerals in the rise of the development issues in foals.

In older foals that are being supplemented, there should be a reduction of the concentrate (feed) to a minimum or completely withdraw ration and should have their food supplemented with hay and balanced mineral supplement and tailored to their nutritional needs (Barr, 2002). As mentioned, there is another etiology that can lead to acquired flexural deformation that occurs when a member suffers a painful stimulus.

The horse, in response to pain does not properly support the member on the soil. Consequently leads to a disuse of soft tissue adjacent to the metacarpal bone and contraction of both.

Regarding the presence of a painful stimulus, in determining the cause of this disease reference is made to certain physical conditions that can cause pain eg: sub-solar abscess, laminitis, the hoof sole hematoma due to exercises on a hard surface, poor support due injury to the contralateral limb, epiphysitis, septic arthritis and osteochondrosis. Although pain can be a factor that triggers the development of acute acquired flexural deformation, other permanent causes of musculo-tendinous

tissue deformation might occur, for example, the flexion contracture of the face of the articular capsule (Barr, 2002). Early diagnosis of FD is important because it increases the success of complete resolution of the lesion, but this may be difficult if the foal is in a pasture next to the mare, especially if the injured joint is the distal interphalangeal, as identification of the lesion can be difficult. Frequent observations on a smooth and hard surface can help to detect this type of injury early (Barr, 2002).

### **1.1.2 Pathophysiology of flexural acquired deformities**

The flexural deformities acquired from the distal interphalangeal joint (FDAID) mainly occur in foals aged 1-6 months old. This injury involves the deep digital flexor tendon (DDFT), because this is part of the solar surface of the coffin bone and is responsible for hyperflexion of the distal interphalangeal joint. Initially, the dorsal hoof wall assumes a more vertical angle relative to the ground and the beads may lose contact with it, especially if the early lesions occur sudden and sharp. When the wall assumes this conformation two sequels occur.

First, there is an overgrowth of lugs, for the lack of wear and contact with the same length as the gripper (dorsal hoof wall). The second sequel it turns to level the gripper, which supports great pressure and suffers excessive wear, which can lead to major changes in the dorsal hoof wall (Barr, 2002).

Changes mentioned above, concerning the conformation of the wall result from the deformation and are not the cause. The FDAID are subdivided into two categories: Grade I and Grade II depending on its severity. Grade I shows that the dorsal hoof wall appears more vertical relative to the ground, but does not extend beyond the perpendicular line between the ground and the hoof wall. In Grade II, the dorsal hoof wall exceeds the aforementioned vertical line from the floor, thus having a worse prognosis than deformations Grade I. The prognosis is also reserved more the longer the period of time that elapses before treatment (Barr, 2002). This classification is useful to describe the deformation and the formulation of the prognosis but does not necessarily dictate the method of treatment below. Thus, the treatment depends on a number of factors, namely the degree of change in the joint angle, foal's age, affected joint and evolution of the lesion. Early treatment favors the FD prognosis, especially those who are involved in some degree of pain. If the primary factor that causes pain able to be identified must first be treated (Auer, 2006). Medical treatment is often limited to symptomatic therapy and analgesia, cut corrective hoof, farriery corrective splints with applying bandages and controlled exercise. Normally this is only successful when the lesion in these treatments has reduced manual handling when the joint on physical examination, ie if you can reset

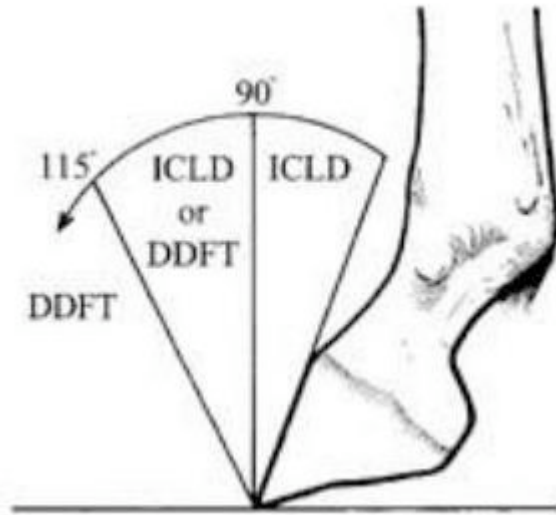


it in a position similar to that of a normal limb conformation to perform extension flexion (mobilization) of joint A. There is no consensus on the recommendation that exercise training during treatment. If the deformation is secondary to a painful cause then the physical exercise should be controlled and the administration of analgesics may be beneficial. (Auer, 2006). Uncontrolled exercise may exacerbate the painful stimulus direction and the foal will contribute to excessive support the contralateral limb. In FDAAID, wherein the foal sometimes only support its weight in the tweezers wall is no benefit in performing controlled exercise on a firm surface, combined with analgesics, which allow the lengthening of DFDP. A young foal should be permitted to move in a small pen to prevent uncontrolled exercise (Barr, 2002).

**Figure 5.** Radiographic image of one of the foals in the study group, affected unilaterally. The image shows the marked deviation in the sagittal plane, while the bottom image is the member observes correctly upright.



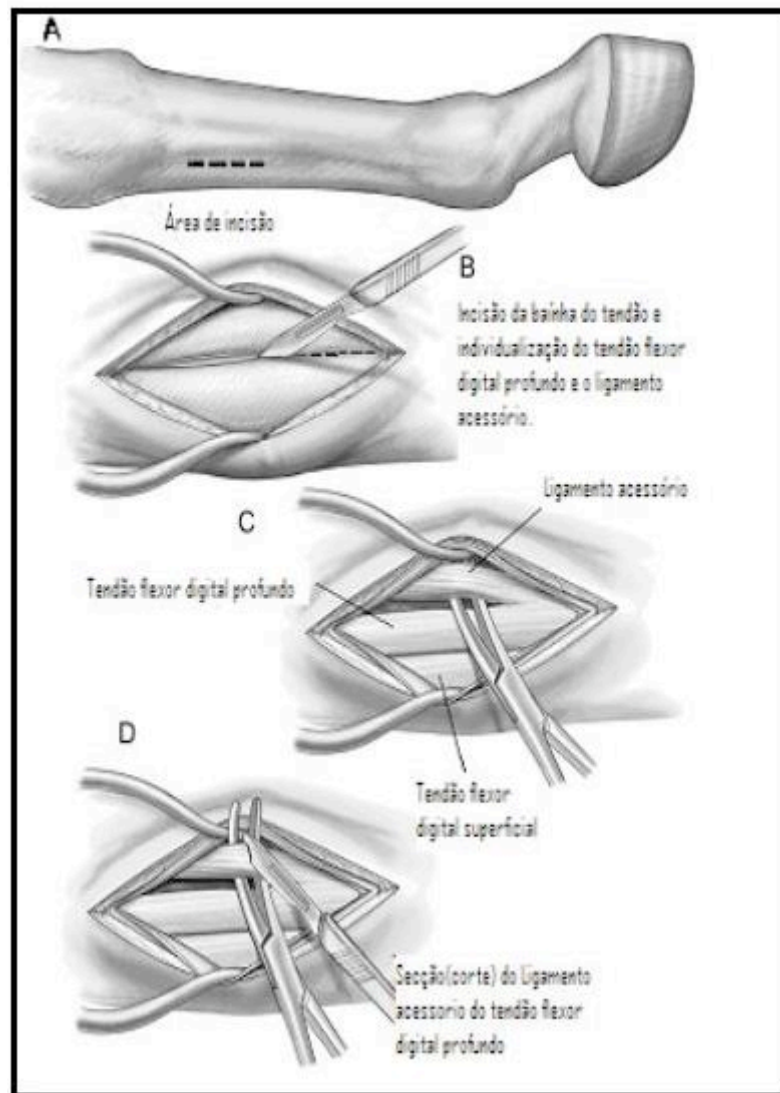
**Figure 6.** Diagram evaluating the angle between the dorsal hoof wall and floor. Indicative parameters for surgical resolution of FDAAID based on the angle between the dorsal hoof wall and the ground. ICLD (Inferior check ligament desmotomy) desmotomy the LATFDP. DFDT (deep digital flexor tenotomy) desmotomy the PRFD. (Management of Congenital and Acquired Flexural Limb Deformities, Stephen B. Adams, DVM, MS and Elizabeth M. Santschi, DVM). 2000.



Only if the medical improvements are not met should this be done by surgical treatment. This should be considered in severe cases of FD and in cases that do not respond positively to medical treatment. Correctively cutting the hooves may have to be conducted with the foal under general anesthesia. Medical treatments can optimize surgical outcomes, despite the poor prognosis of cases involving surgery. The desmotomy accessory ligament of the deep digital flexor tendon (LATFDP) is used as surgical treatment of FDAID. Surgery is usually performed under general anesthesia with the horse in lateral or dorsal position. At this point, corrective hoof trimming and possible application of extensions tweezers. The purpose of corrective cast will establish a normal axis between the pastern and hoof. A technique of desmotomy LATFDP guided by ultrasonographic examination has also been the subject of description in stationary horses subject to sedation. The author reported that this surgical technique had a higher degree of success in restoring normal hoof conformation in younger foals (mean age 6 months) compared to the group of older foals (mean age 12 months), although the age at time of treatment did not affect the end result cosmetic surgery (Auer, 2006). Often, improvement is shown of FD postoperatively, as confirmed in the following days. The surgical areas are maintained with dressing and bandage until the removal of the skin sutures and exercise, while encouraged, should be limited (Auer, 2006). In horses whose sport is relatively limited, and reduced level of demand, the prognosis is good. In a study conducted in the UK and published in 1980, 86% of horses treated before reaching 1 year of age were subsequently used in the sport for which they were acquired. In the case of animals treated after 1 year of age there was a success rate slightly lower 78%. It was reported by Fackelman (1980), a good prognosis in correcting mild cases, but poor response to surgery in more severe injuries. Stick et al. (1992) reported that in Standardbred foals (trotters) undergoing surgical treatment they were able to achieve their full athletic potential, but considerably improved the prognosis in cases of foals treated as soon as possible. The outcome for foals

whose treatment was applied after 8 months of age was not favorable. In FDAID Grade II, the surgical technique should be tenotomy of DFDP as in horses that were subject to the desmotomy LATFDP and showed no improvement with this technique. This procedure is used only for conservation of animal life, since, in functional terms, the prognosis is extremely reserved (Auer, 2006).

**Figure 7.** Schematic representation of the desmotomy LATFDP. Adapted image (Auer, 2002).



## **1.2. Mechanotransduction**

The mechanotransduction is the process by which cells perceive mechanical stimuli and convert them into cascade of cellular and molecular events that alter its structure. The cellular components involved include the extra-cellular matrix (ECM), the cytoskeleton, integrins, G proteins, tyrosine kinase receptors and calcium channels (Wang, 2005).

According to the Theory of Tensegrity (Ingber, 1999), the forces exerted by the MEC on the cell are in equilibrium with the forces exerted by the cell on this. These forces are transmitted to the cell via integrins, cell junctions and also by the MEC. The mechanical forces applied on the cell surface are transmitted directly to the cytoskeleton and cause changes in the structure thereof (Wang & Ingber, 1995). Thus, cytoskeletal changes due to application of mechanical forces can initiate a complex signal transduction cascades within the cell through activation of integrins, receptor stimulation of G proteins and receptor tyrosine kinase (Wang, 2011).

Is that the actin cytoskeleton has a role in mechanotransduction because it transmits and modulates the voltage between the ECM, focal adhesion sites and integrins. Subsequently, this leads to conformational changes in integrins, G proteins and ion channels composition where such changes stimulate membrane receptors and induce complex biochemical cascades (including activation of transcription factors), with subsequent protein synthesis, gene expression and differentiation cell (Wang, 2001).



The lack of use of a tendon, also called deprivation deformation due to immobilization or disuse, is responsible for degenerative tendon. In fact, the disuse of the tendon leads to a marked decrease of elasticity or deformability, as studied in patellar tendon of rabbits subjected to deprivation of use for 3 weeks (Yamamoto et al. 1993).

In response to the mechanical forces to which they are subject, the tendons can promote alterations in their metabolism as well as their mechanical and structural properties.

For example, in response to an appropriate physical training a tendon increases its cross sectional area and fibroblasts enhance the production of type I collagen, leading to an increased elasticity of the tissue (Suominen et al. 1980; Michna & Hartmann, 1989; Langberg et al. 2001; Tipton et al. 1975).

However, inappropriate training leads to injury and tendinopathy with increased inflammation mediators such as Prostaglandin E2 and Leukotriene B4 (Li and such., 2004, Wang et al. 2001).

The ability of connective tissues, such as tendons, present to change its structure in response to mechanical stimuli is designated by mechanical adjustment of tissues.

This adaptation is performed by cells. However, the mechanisms by which mechanotransduction cells perceive the mechanical forces and convert them into biochemical signals that ultimately lead to adaptation physiological or pathological tissue, are still not fully understood and is the subject of study Mecanobiologia molecular, cellular and tissue involved in these processes (Wang, 2005). Fibroblasts specific tendons, and are designated by tenoblastos tenócitos. They are the dominant cell types and those responsible for these mechanical adaptations that a tendon is capable of performing. The fibroblasts are responsible for the protein synthesis of the extracellular matrix (collagens, fibronectin and proteoglycans) also having the capacity to alter the expression thereof (Banes et al. 1999; Ralphs & Benjamin 2000; Kjaer, 2004).

Various types of glycoproteins present in the ECM including fibronectin and tenascin-C.

The tenascin-C contributes to the mechanical stability of the ECM through its interaction with collagen fibrils (Elefteriou et al., 2001). The role of fibronectin is related to the recovery from injury, and its synthesis increased in cicatrization

processes (Józsa et al. 1989a; Williams et al. 1984). The processes that lead to these adaptive cellular responses, are the domain of mechanotransduction. From the mechanical standpoint, the ECM transmits the forces exerted on the cells and accumulates and dissipates the energy of elastic forces induced. Mechanical deformations of the ECM are transmitted to the actin cytoskeleton and lead to remodeling thereof (Wang, 2000, Wang et al. 2001).

The cytoskeleton to control cell shape affects their motility and is intermediate in various cellular functions, including DNA and protein synthesis (Janmey, 1991). It is composed of microfilaments, microtubules and intermediate filaments and has a role in mechanotransduction (Ingber, 1991).

The microfilaments are associated with a large number of proteins, forming a continuous and dynamic connection between practically all intracellular structures.

The cytoskeleton responds to extracellular forces, participates in transmembrane signaling and provides a network for the organization or translocation of signaling molecules (Wang, 2011).

### **1.2.2. Mechanobiology of the bone tissue**

Bone tissue, such as tendons, are also subject to a variety of mechanical loads applied during daily activity.

The surgeon Julius Wolff German in 1892 proposed that, in order to optimize the load capacity, bone mass can adapt its three dimensional structure and load conditions to which they are subjected, this process being driven by mechanical shear forces. The osteocytes are the principal cells in orchestrating this regulation structure and biomechanics of bone mass, which is achieved through the process of bone remodeling. They are the most abundant cells in adult bone, exhibit a characteristic shape dendrítica radiating processes starting from the cell body, housed in tubules, arranged in different directions. These processes form a network through intercellular junctions involving osteocytes, cells of the periosteum and bone marrow. Through this unique three-dimensional network, osteocytes are anatomically placed in a position not only to primary percepcionarem deformations caused by stresses to the bone printed but also to produce and transmit a response to neighboring cells through cell communication signals (J. Klein-Nulend et al., 2009).

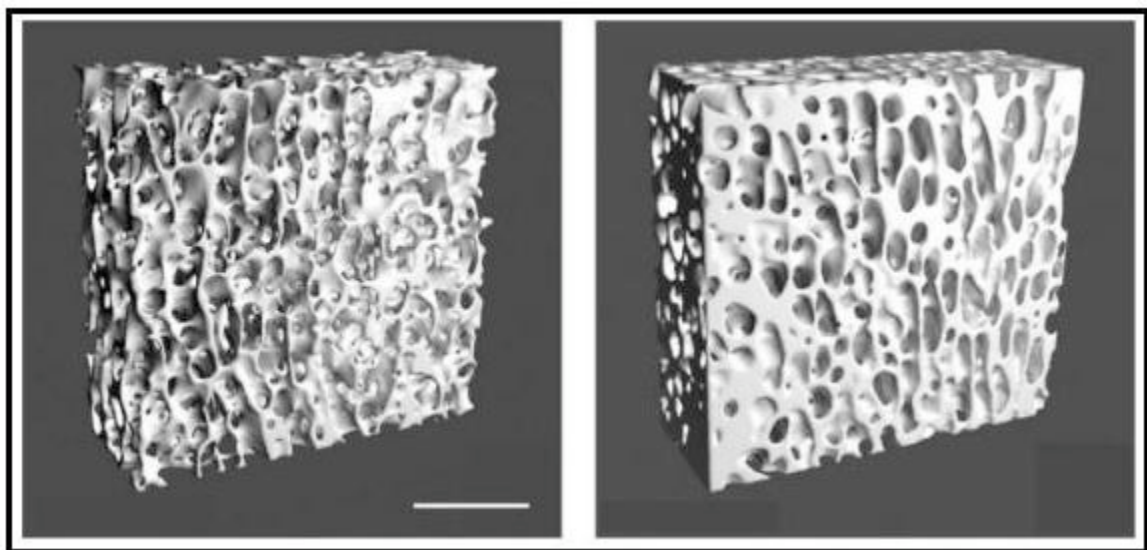


Currently, it is known that when the bones are subject to loads, the resulting deformation will cause the thin layer of liquid surrounding the interstitial network of osteocytes described above, the bone regions to flow under high pressure to regions of the bone under low pressure.

It is this movement of liquid and which triggers a response that is the production of signaling molecules. These regulating bone resorption by osteoclasts held executed and bone formation by osteoblasts, leading to Nulend, et al. 2009). However, Vatsa et al. 2008, proposed that osteocytes could perceive tensions bone matrix directly and that, accordingly, the cell shape, cytoskeletal alignment and distribution of adhesion sites exhibit patterns corresponding to the tensions that were being applied to the bone. Wang et al. 2008, developed a model to evaluate how integrin adhesion complexes present along the dendritic processes of osteocytes could detect mechanical deformation to soft tissue level. It was shown that these cellular processes are adhered to in these integrins canalicular projections may respond to tension forces below 15 picoNewtons. As observed and reported to the tendons, here too the cytoskeleton plays a crucial role as structure sensitive and responsible response to physical stimuli.

The cytoskeleton is involved in processes like mechanoperception and determines the material properties of the cell (eg stiffness) which are the same material properties of the objects, and which influence the effect resulting from the application of voltages of different values.

**Figure 9.** Three-dimensional reconstructions of trabecular bone of the distal femur of sheep. Left image: Control. Right image: sheep subjected to vibrations of low magnitude (0.3g) and high-frequency (30Hz) for 20 minutes daily for a year. Adapted from Rubin et al, 2002



Studies in sheep by Rubin et al., 2001, show that animals were subjected to 20 minutes of daily mechanical vibration of low magnitude (0.3 g) and frequency of 30 Hz (cycles per second) for one year improve whereas the observed deformations during normal locomotion are between 500-frequency (10-100 Hz) may stimulate bone growth and inhibit disuse osteoporosis. Bacabac et al. 2008 also demonstrated that the cellular response to mechanical stress is related to the material properties of the cell, which implies that the bone cell response to mechanical stress is related to the properties of the cytoskeleton. Apparently osteocytes elastic require less mechanical force to produce an answer, relative to more rigid cells. This indicates that differences in mechanosensitivity between osteocytes and osteoblasts can not only be related to the elasticity of the cell, but may also be related to specific properties of the cell, ie, presence of receptors or ion channels in the membrane or alter the cells as their material properties in relation to the deformation.

However, although some mechanisms that make osteocytes responsive to such stimuli are not fully elucidated, these regulate bone remodeling by directing the function of osteoblasts and osteoclasts in response to these mechanical environmental conditions (intensity and frequency of stress).

### **1.3. Environmental Conditions of the Stud**

As mentioned earlier in this chapter the FD described appeared to coincide with the implementation of six wind turbines (WT) on land adjacent to the stud. The turbines, whose function is to transform kinetic energy of wind into mechanical energy and consequently into electrical energy. A wind turbine consists of the following elements:

- A tower, which allows the wind turbine to raise more regular winds in the atmospheric boundary layer.
- A ship or nacelle containing the mechanical system.
- A shaft allowing rotation of the blades that transmits the mechanical power to the electric generator.
- The blades that absorb kinetic energy from the wind.

Its implementation has been a significant development in Portugal (third in world per capita and second in relation to PIP (gross domestic product) (<http://cleantechnica.com/2011/04/11/>)).

Each WT varies in size and ability to produce energy from a few kilowatts to several megawatts. We find them singly or in wind farms, and the largest of these in Portugal (Ventominho SA) has 120 wind turbines.

They are usually installed in non-residential areas and low population density, thus bringing no apparent environmental impact.

The Stud studied in this work, is adjacent to a wind farm comprising 9 turbines REpower, model MM92 2 MW power unit. The TE rotor having a diameter of 92 m and its height is 80 m.

### **1.3.1 The noise generated by WT**

The noise and the sound from a strictly physical point of view, are the same magnitude. The difference lies in the quality perceived by acoustic receivers. This observation leads us to define noise as unwanted sound, and physically, both are sound pressure waves that propagate in an elastic medium (liquid, solid or gaseous).

The sound is detected by ear through a mechanical process in which the sound waves are converted into vibrations on the ear and subsequently into electrical impulses which affect the brain and are interpreted by this. (Goeltzer, 2001).

At this hearing process, the change of the vibrating pressure on the eardrum leads to stimulation of the auditory nerves, which have various calibers corresponding to the uptake capacity of different frequencies (Kinsler, 1982).

Noise can affect the persons physical, psychological and / or social perception, because it can cause hearing loss, communication interference, discomfort, fatigue and reduced work capacity (Maia, 1992).

Acoustics is the science of sound and noise. He also devoted himself to the study of the propagation of sound waves through gaseous, liquid and solid, and its interconnections with the human.

The characterization of the sound is done by frequency, wavelength and amplitude. The higher the frequency, the more cycles of oscillations occur over time, designated period (T). The low frequency bass sounds are unlike those produced by higher frequencies that produce sounds.

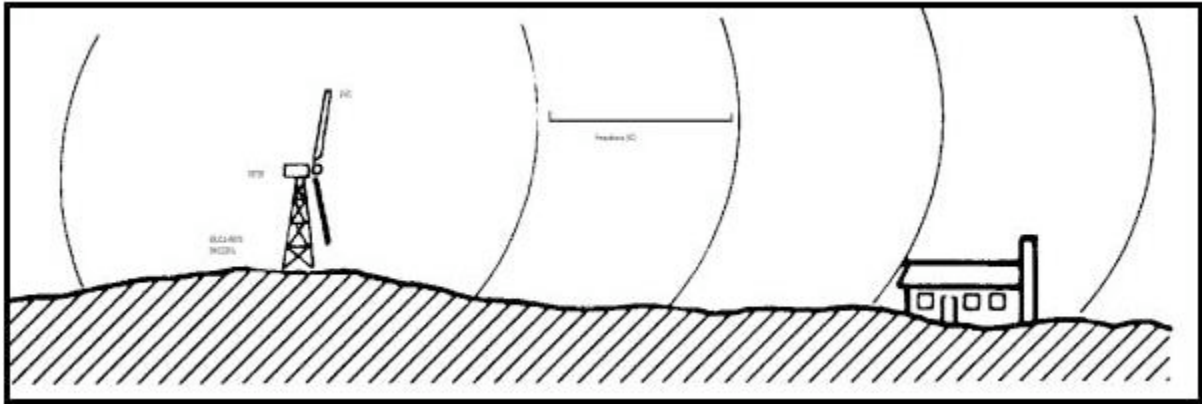
Below 20 Hz (lower limit of hearing for humans) are the Infrasound and above 20 kHz (the upper limit of the audible range) ultrasonic sensors (Matthew, 2008).

The frequencies of the noise produced by wind turbines vary from low values that are sometimes audible, to high values belonging to audible range of values (Kelley et al. 1985).

Although the distance phenomenon is beneficial in reducing noise levels, wind can increase the spread of noise in one direction and prevent in other directions.

A feature of the noise produced by wind turbines is that this is delivered continuously during the day and night. By contrast, for example, aircraft or road noises vary markedly depending on the time of day.

**Figure 10.** Schematic representation of sound waves emitted by the WT and its spread.



The WT generate sound through two mechanisms: mechanical and aerodynamic. The aerodynamic noise is related to the geometry of the rotor blades and the environment where the turbine is located. Mechanical noise is associated with the sound made by the wheels of the rotor.

Exposure to low frequency noise can cause vibration in the human body.

Exposure to vibration can reach certain human tolerance limits and may be a source of psychological and physiological deficiencies (Smith, 2006).

The vibration is a mechanical oscillation that varies over time in a mechanical or biological condition of equilibrium, in which the average of its movement will tend to zero and which may be present movements of translation, rotation, or both (Brammer, 2004).

Humans exposure to large magnitude vibration is often associated with trauma leading to physical damage (e.g. fracture, bleeding, tissue lacerations). When the vibrations occur at moderate magnitudes physiological effects can include chronic pain, especially involving the spine.

In animals (dogs and rats) short intense vibrations led to death. The internal lesions observed postmortem were consistent with shock bodies against the chest and also due to the resonance phenomenon of heart and lungs (Griffin, 1990)

Some authors describe the physiological effects caused by vibration induced by Infrasound and low frequency noise such as Vibroacoustic Disease (Alves Pereira and Castelo Branco, 2002).

### **1.3.2. WT vibration propagated through soil**

The TE generate mechanical vibrations. These can be detected by broadband seismometers buried in the ground to several tens of kilometers away from the wind farms (Styles P. et al, 2011).

These physical phenomena that occur during the operation of TE deserve to be the subject of detailed study, to ascertain whether there is any relationship between these and flexural deformations and other lesions observed in foals at the stud farm study.

This project aims to study the possible correlation between the emergence of FD by the effects of mechanotransduction phenomena induced by physical forces that produce the TE in the environment where the horses live.

## **CHAPTER II - MATERIALS AND METHODS**

### **2.1. Animals**

We studied 11 horses (*Equus caballus*), all Lusitano breed, aged between 0 and 48 months, belonging to a stud farm located near Lisbon Torres Vedras (Table 1).

In this group of animals, 5 were female and 6 were male. All horses studied had flexural deformation of the distal interphalangeal joint of the forelimb.

To exclude possible genetic causes, two animals were acquired by the owner from other stud farms with no history of flexural deformation.

On April 17, 2008, when these two animals were purchased from the stud they did not exhibit any flexural pathology.

However, after a few months, both developed the same symptoms as the remaining affected foals. They were transferred to stabling at the College of Veterinary Medicine on 22<sup>nd</sup> of October on humanitarian grounds.

All animals were fed a commercial diet suitable for horses. There was no change in relation to conditions of housing or the exercise regime to which the stud horses were subjected period prior to the appearance of cases of FD.

**Table 1.** Group of horses studied

Horses studied						
Name	Sex	Birth Date	Father	Mother	Provenance	Notes
Canela	F	26.02.2007	Operario	Juvita	Born in the stud	
Desplante	M	02.04.2008	Importante	Vassoura	Aquired from Hd das Silveiras	17.04.2008
Dondoca	F	04.04.2008	Uranio	Escalabitana	Aquired from another breeder	03.06.2009
Espartaco	M	02.05.2009	Zircao	Vassoura	Born in the stud	
Engenheiro	M	17.05.2009	Zircao	Zizi	Born in the stud	
Furacao	M	05.02.2010	Zircao	Zona	Born in the stud	
Fadista	F	12.02.2010	Zircao	Zaza	Born in the stud	
Formosa	F	15.02.2010	Zircao	Bambina	Born in the stud	
Faneca	F	18.04.2010	Zircao	Juvita	Born in the stud	Euthanasia 10.11.2010
Fundi	M	27.04.2010	Zircao	Vassoura	Born in the stud	
Flamengo	M	30.05.2010	Zircao	Zizi	Born in the stud	

**Figure 11.** Foals born in 2011 near the progenitor at stud. The red arrows indicate the affected joints and the black ellipses the presence of wind turbines



## **2.2. Examinations carried out**

### **2.2.1. Anamnesis**

The clinical history of the equine stud has been thoroughly investigated, particularly nutrition, exercise, the management, the conditions of housing and the clinical condition of horses.

We studied the remaining horses at stud residents born before 2008 and those who remain within the stables.

### **2.2.2. Clinical examination**

The performance were evaluated by a complete orthopedic examination held in the assessment of locomotor (test static and dynamic test) with the use of additional diagnostic methods, such as X-ray and ultrasound examination, the results of which are described further in this work. The examination of the locomotor system was conducted in two parts: static test and dynamic test.

During the static test horses were observed in station, without any restraint beyond a fit for purpose halter, on a flat surface (Figure 12).

We evaluated the conformation through palpation of the limbs and hooves. The presence of pain was assessed on the hoof by means of a clamp shells which allowed the application of force in corneal tissue (Figure 13).

We then proceeded to manipulate (flexion and extension) joints evaluating reactions of pain and range of motion of the same.

In the second part of the examination of the motoring system, the dynamic examination, the horse was seen walking in its three natural gaits: walk, trot and canter.

Firstly the gait of each horse was observed whilst walking in a straight line, and then trotting. Subsequently, each animal was lunged in a circle with a variable diameter which evaluated and quantified lameness.



**Figura 12.** Static examination. The horse is to be observed in a flat and smooth surface without the use of sedation to be observed conformation.



**Figure 13.** Evaluation of the hoof using forceps. We considered the reactions of pain displayed by the animal.





### **2.2.3. Goniometry. Measuring the angle between the dorsal hoof wall and the soil**

The distal ends of the forelimbs were subjected to measurement with a goniometer which is the instrument that allows determination of the angle between the dorsal hoof wall and the ground (Figure 14).

With the animal stationary on a flat surface, the apparatus was applied to the wall of the hoof for said angular value reading. In spite of the individual variability of the conformation of horse hooves on average, the angle for a wall forelimb should be between 50 and 55 degrees.

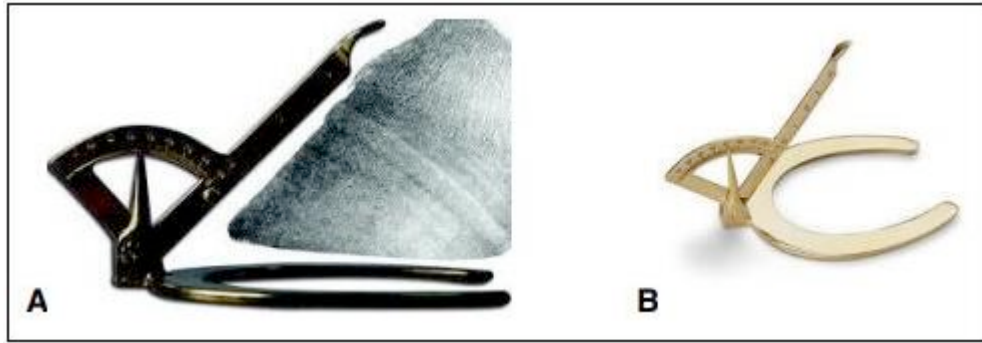
### **2.2.4. Radiological examinations**

Radiological examinations were performed on all animals studied and the distal ends of the forelimbs evaluated. We made the following projections (or plans): lateral-medial and dorsal-palmar. These examinations were performed at Stud ampoule with a portable generator rx, described below. To carry out this examination, it was not necessary to pharmacologically subdue horses. The hoof was held flat and smooth in an area where the luminance was decreased to achieve a good focus on placement of the area of interest.

To obtain a higher quality picture and diagnostic value we proceeded to the collimation of the radiation beam. The cassettes were placed in parallel relation to the member and the closest. The operator put the ampoule perpendicular to the x-ray structure and proceeded to obtain radiographs, taking all necessary safety precautions regarding the radiation within the safety practices of the operator and helpers present at examination.

The X-ray equipment used was as follows: Generator monobloc high-frequency: Toshiba Tube RX Focus: 2x2mm; voltage: 40 to 110KV in brackets 2KV; earners mA: 25, 28, 30, 35, 40, 45, 50, 60mA Samil brand, model TW 116 and a scanner image Vetray brand, model CR 35V (Figure 15).

**Figure 14. A and B.** Goniometer. Instrument for measuring the angle between the dorsal hoof wall and the soil, as exemplified in the Figure.



**Figure 15.** Scanner used in the processing of radiographic images and vial radiation generating.



### 2.2.5. Sonographic examinations

Scans were performed of the distal forelimb tissue of the affected animals. For this examination, the trichotomy of the palmar aspect of the limb in the area between the carpal joint and the start of the heel of the hoof was covered. The area was washed with warm soapy water, and ethyl alcohol 70 ° gels suitable for the purpose applied, in order to obtain the best possible contact between the probe unit and the skin of the limb of the animal to better capture images.

The soft tissues of the region were observed sonographically, with particular emphasis on digital flexor tendons (superficial and deep), the suspensory ligament of the fetlock and digital accessory ligament of the deep flexor tendon.

During the test, a probe was applied on the member perpendicular to the longitudinal axis of the distal end of the member and parallel to it, in order to obtain images in transverse and longitudinal structures studied.

We used an ultrasound device Aquila Vet with image snapshot digital image through a probe 7.5 Hz Cineloop Technology (DRII) with software for veterinary Ref: ESA-97154410853.

#### **2.2.6. Measurement of Cortical Bone**

In animals of the study group, born between 2010 and 2011, we evaluated the thickness of the cortical bone at the level of the third metacarpal, dorsal and lateral in both forelimbs by conducting a quantitative examination ultrasound. (QUS). This is a non-invasive technique for assessing the properties of cortical bone.

Through this method, the propagation time of the sound waves is computed to determine the velocity of sound waves and the time it takes to go through the bone and thus know its thickness.

The method was used in the third metacarpal bones of horses to determine the speed of sound through the bone, and the maximum speed would be when the cortex was thinner (Jeffcott and McCartney, 1985).

Glade et al. in 1986 reported that this method is portable and simple but reproducible in which the variation of the soft tissue may lead to some degree of error. The speed of transmitting ultrasound is relatively accurate (1.5%) and is influenced by the bone density and architecture (Nicholson et al. 2001).

For this purpose three measurements were performed on each animal, with time intervals of 2 months.

For this exam the animals were not subjected to any pharmacological sedation or tranquilization.

In order to ensure the horses remained calm during the examination, thus decreasing the likelihood of getting injured and also for the safety of operators and equipment, fit for purpose restraints in the form of a 'crush' as used in the stud (Figure 16).

The probe of the apparatus was applied with firm pressure on the middle portion of the metacarpal bone (approximately 10 to 12 cm distal to the carpus).

We used an ultrasound device QUS (Sunlight EQUUS, Sunlight Medical, Israel).

The reference values for this study were those determined for the Lusitano breed by Fradinho et al, 2009.

**Figure 16.** Animals inside the 'crush' for exams.



### **2.2.7. Computerized Axial Tomography (CAT)**

The test was performed post-mortem, from the member removed at necropsy of an animal, the premises of the Hospital of the College of Veterinary Medicine.

During this examination the members were placed inside the machine in the same position they would observe normally.

### **2.2.8. Desmotomy and biopsies of the accessory ligament of the deep digital flexor tendon**

In 2008, two foals had desmotomy of the accessory ligament of the deep digital flexor tendon under general anesthesia. No tendon tissue was harvested as it was assumed that the problems resulted from an isolated case. Only later did it become apparent that similar symptoms would manifest in all animals born later. Both instances were held at stud (Figure 18).

The animals were subjected to 12 hours of fasting prior to the intervention. The doping was performed with acepromazine. The area on the side of the forelimb, in the middle region of the third metacarpal was shaved and surgically prepared with antibacterial solution iodine and alcohol. Surgery was performed using the technique described in Chapter I, carried out under intravenous general anesthesia with ketamine (2.2 mg / kg) and diazepam (0.1 mg / kg). Tissue was harvested for histology. On completion, the limbs were appropriately sutured.

A broad spectrum antibiotic (250mg/ml 200mg/ml penicillin G procaine + dihydrostreptomycin at a dose of 0.04 ml / kg once a day by intramuscular) and non-steroidal anti-inflammatory drug (at a dose of 2 Phenylbutazone, 2mg/kg once a day, orally) for 7 days was administered.

Three animals were subjected to biopsies (LATFDP and DFDP). They were tranquilized with acepromazine and later sedated with detomidine. A biopsy was

performed in the same region as desmotomy described above and with the same care for the preparation of the area of intervention. The foals were anesthetized by subcutaneous infiltration with 2% lidocaine; tissue biopsies were taken for histopathological analysis. After this intervention, broad spectrum antibiotics and anti-inflammatory agent were also administered for 7 days (Figure 19).

### **2.2.9. Histopathological analysis**

The material collected for biopsy in desmotomy mentioned in 2.2.8 as well as obtained at autopsy was processed according to routine histopathology techniques for their age their condition (Figure 16). At necropsy fragments of the following tissues and organs were collected:

- Skin
- Central nervous system
- Lung
- Spleen
- Liver
- Esophagus
- Stomach
- Pancreas
- Large intestine
- Small Intestine
- Heart
- Kidney
- Bladder
- Thyroid
- Adrenal Glands
- Ovary
- Eyeball
- Smooth muscle tissue

- Striated muscle tissue
- Tendons of the distal forelimb (deep digital flexor tendon and suspensory ligament and superficial billet / III interosseous muscle)
- Biopsy tissue samples from the deep digital flexor tendon and accessory ligament were also obtained from five foals (see next section).

**Figure 17.** Autopsy of the horse named "Faneca" in the College of Veterinary Medicine autopsy room.





**Figure 18** - Equine with the name of "Desplante" subjected to a desmotomy LATFDP under general anesthesia.



**Figure 19** - Equine with the name of "Engenheiro" subjected to a biopsy performed under sedation and local anesthesia.



The tissue samples were placed in buffered formalin 10% by volume ten times greater than that of the piece. After a minimum of 24 hours fixing, processing and embedding in paraffin the tissues were carried out in an automatic tissue processor Leica ® PD 1020 according to the following protocol:

A - Ethanol A-70 for 1 hour.

B-Ethyl alcohol 95 ° for 1 hour and 30 minutes.

C-Ethanol 95 ° for 1 hour and 30 minutes.

D-Ethanol absolute for 1 hour.

E-absolute ethyl alcohol for 1 hour and 30 minutes.

C-Ethanol absolute for 1 hour and 30 minutes.

G-Xylene for 1 hour.

H-Xylene for 1 hour.

I-Xylene for 1 hour.

Inclusion in Histosec J-Merck ® at 65 ° C for 2 hours.

Inclusion in L-Histosec Merck ® at 65 ° C for 2 hours.

Block cuts were performed on paraffin rotary microtome Leica ® RM 2135 and slide Leica SM 2000 ®

The hematoxylin and Erythrosine (H & E) was performed on all tissues collected.

Also, there were stains Van Gieson, Congo red according to the protocols described below.

The H & E staining was performed according to the following protocol:

A-Xylene for 15 minutes.

B-Ethanol absolute, three passages.  
C-90 ethyl alcohol, three passages.  
D-Ethanol 70th, three passages.  
E-distilled water, rinse well.  
F-Ehrlich's hematoxylin for 10 minutes.  
G-distilled water, one pass.  
H-hydrochloric Alcohol 1% (70 ° alcohol), a passage.  
I-distilled water, one pass.  
J-common water until dark, about 2 minutes.  
L-Erythrosine for 2 minutes.  
M-distilled water, one pass.  
N-Ethyl alcohol 70, three passages.  
The Ethanol-90, three passages.  
P-Ethanol absolute, three passages.  
Q-Xylene for 5 minutes.  
R-Mount synthetic resin (Entellan ®).

Van Gieson staining was performed according to the following protocol:

F-Weigert's hematoxylin for 10 minutes.  
G-Wash in warm water.  
I-Wash in warm water.  
J-van Gieson for 3 minutes.  
L-distilled water, rinse well.  
M-Ethanol 70th, three passages.  
N-Ethyl alcohol 90 °, three passages.

O-The-Ethanol absolute, three passages.

P-Xylene for 5 minutes.

Q-mount in synthetic resin (Entellan ®).

The Congo Red staining was performed according to the following protocol:

C-90 ethyl alcohol, three passages.

F-Congo Red solution for 5 minutes.

G-Differentiating with potassium hydroxide solution for 3 to 10 seconds.

H-wash with distilled water.

I-Mayer's hematoxylin for 3 minutes.

J-Wash with running water.

L-Ethanol 70th, three passages.

M-90 Ethanol, three passages.

N-Ethyl alcohol absolute, three passages.

O-Xylene for 5 minutes.

P-Mount synthetic resin (Entellan ®).

#### **2.2.10. Noise measurements noise**

The noise was measured in the stud farm pasture where the affected animals remain on a daily basis, on land adjacent to it, and the site of a wind turbine.

We used a sound level meter Brüel and Kjaer Pulse with 4 channels (Model 2827-62), a microphone, 1-inch, free-field (Model 2570), a preamplifier (Model PRM902) and a calibrator (Model CAL200).

#### **2.2.11 measurements of vibrations soil**

Studies have been conducted measuring mechanical vibrations. These were performed by ICIST / IST (Institute of Structural Engineering, Territory and Construction, IST, Technical University of Lisbon) at the request of the Faculty of Veterinary Medicine.

The purpose of the measurements was to characterize the mechanical vibrations induced by the operation of wind towers in different locations of the holding.

The data recording was performed on two separate occasions: March 22, 2012 and June 25, 2012.

The experimental procedure adopted was the collection of records triaxial vibration at different sites within the holding well as in some locations outside of it, closer to or away from the wind tower.

The instruments to detect vibration were installed on the ground, with digital data snapshots of vibration with varying durations, close to 1 minute.

Records of vibration were made with the use of digital recording units triaxial accelerations (Kinemetrics brand, model ETNA (with internal sensor Episensor)), similar to that shown in Figure 20. On the first day of data collection three different units were used; in the second stage only one unit is used. The most relevant features of unit used for recording vibrations are briefly indicated in the following list:

- Episensor triaxial sensor with the following specifications:
- the dynamic range exceeding 135 dB
- the sensor bandwidth from DC to 200 Hz
- The linearity  $<1000 \mu\text{g/g}^2$
- the hysteresis  $<0.1\%$  of full scale
- The cross-sensitivity of less than 1% (including misalignment)
- the full-scale hardware selectable between 0.25g to 4g

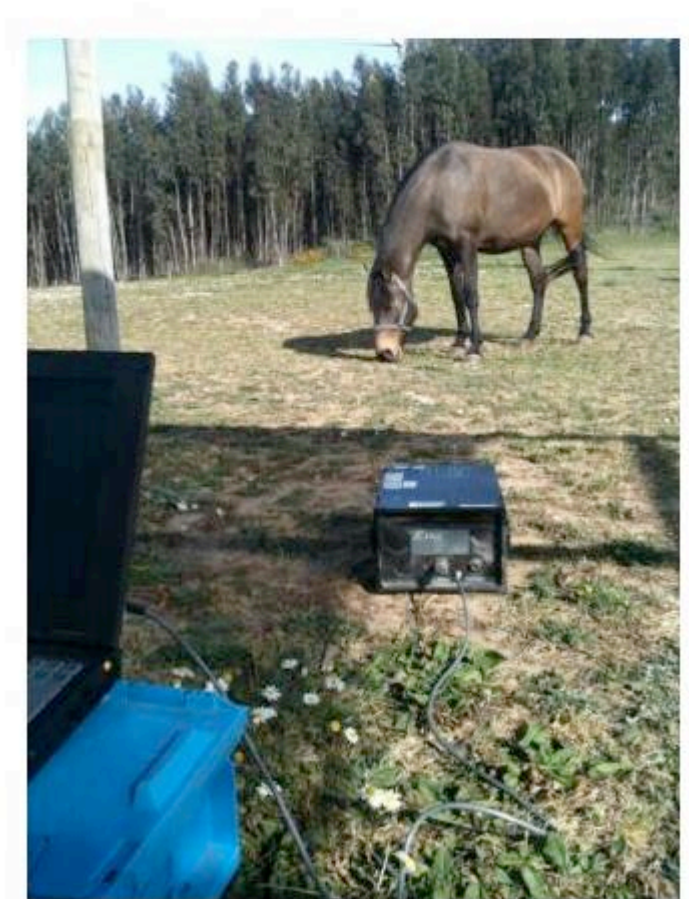
Drive signal conditioning, digitization and registration with the following specifications:

- storage of records in internal PCMCIA card conditioning and filtering analog signal, particularly by low-pass filtering with cut to 80% of the Nyquist frequency and 120 dB attenuation at Nyquist frequency
- the analog-digital conversion performed with 18-bit resolution
- storage of records in internal PCMCIA card.

The unit used was configured with a full-scale 1g, corresponding a resolution on acceleration, after scanning, 0.0008 cm / s.



**Figure 20** - Triaxial unit of acceleration registration



## **Chapter III – RESULTS**

### **3.1. History and clinical examination**

From the year 2008, we observed the appearance of FD at the equine stud. In this stud, horses are fed a balanced diet adapted to each age group, which was considered nutritionally correct and balanced from the standpoint of minerals and micronutrients. The stud farm management in general was considered appropriate: The foals are born into closed pastures and then remain with the dams until these are separated at weaning which occurs at about 6 months. They continue to graze in the pasture up to 3 years, when selection and sell off occurs. These pastures have dimensions appropriate to the number of animals show no steep slopes and soil is suitable for a natural pasture of good quality. The mares remain permanently in the pasture for reproductive purposes and stallions/colts are stabled in suitably ventilated and sized boxes from the point of view of hygiene and animal welfare.

We investigated the clinical histories and records of developmental orthopedic injuries at the stud, as well as a few young horses that live there and were born before the change of environmental conditions. Regarding the history of changes observed in the stud, there were no similar orthopedic injuries to those described in this work, in the years prior to the introduction of the TE in neighboring lands.

All horses born before 2008 on the stud (and those that still remained) were observed and found to be properly upright, i.e. not exhibit FD.

All animals were properly immunized against Equine Influenza and Tetanus, and the worming regime was sufficiently frequent. All animals had good body condition, healthy and shiny coat, physiological parameters (heart rate, and adventitious breath sounds, sounds gastrointestinal mucous membrane color and capillary refill time) normal and good temperament.

The orthopedic examination, ie, the evaluation of musculoskeletal system, as mentioned, was conducted in two parts: static test and dynamic test.

Regarding the observed conformation, all horses in the study group had FD of the forelimb of varying degree, which occurred bilaterally in some animals. The forelimb showed marked deviation in the sagittal plane. The wall assumed a walled form, that is,

the beads become too long and relatively more perpendicular to the ground acquiring a geometry similar to a parallelepiped wall (Figure 21). The dorsal area of the coronet was abnormally convex due to persistent hyperflexion of the distal interphalangeal joint. In some animals there was excessive wear of the caliper wall as a consequence of FD.

**Figure 21** Foals study group. Conformation typical of this wall FD



With regard to the palpation and manipulation of the limbs, it was found that one could not proceed to the manipulation (flexion and extension) of the distal interphalangeal joint with the same amplitude as the joints of the limbs that were not affected, particularly in relation to the length of said joint. However, there were no signs of pain during the test, even when the clamp palpation of walls, thus excluding the presence of abscesses or bruising of the sub-solar wall. However, in some animals exhibiting increased wear of the clamp (due to the FD), there was some sensitivity when applying pressure to the clamp shells.

In horses whose schooling enabled it, the gait was observed, to identify lameness. In horses that had bilateral SCD, the gait was so difficult that the animal showed great reluctance to move. The inability to feed normally and have access to a drinker, significantly reduces their well-being and quality of life.

As mentioned in the previous chapter, to exclude the possibility of a genetic cause for FD, the Company purchased two animals of different genealogy to said stud. These horses also developed a confirmed diagnosis of FD.

Regarding the nece stabled on the premises of FMV, the FD showed a significant although moderate, improvement, (Figure 22). At the date of the images shown, the animal had not been subjected to any medical or surgical treatment.

Within the stud, the two acquired fillies were moved to grassland farthest from their original pasture and these two fillies showed significant improvements without resorting to medical or surgical treatment.

**Figure 22.** Figure 22 - Equine with the name of "Fundi" on the premises of the College of Veterinary Medicine

A - Image of horse, by the water cooler, at Stud in September 2010.

B, C and D images obtained in May 2011. The left forelimb shows improvement in the sagittal plane.





### 3.2. Goniometry. Measuring the angle between the dorsal hoof wall and the soil

All horses in the study group showed angles between the dorsal hoof wall and the soil, higher than the normal value exceeds some even  $90^\circ$  (Figure 23).

### 3.3. Radiological examinations

Radiological examinations performed at the distal forelimb revealed that the phalanges had a noticeable deviation in the sagittal plane, with subluxation of the distal interphalangeal joint.

Bone remodeling and degenerative lesions of the third phalanx and the distal interphalangeal joint was also observed (Figure 24).

### 3.4. Sonographic examinations

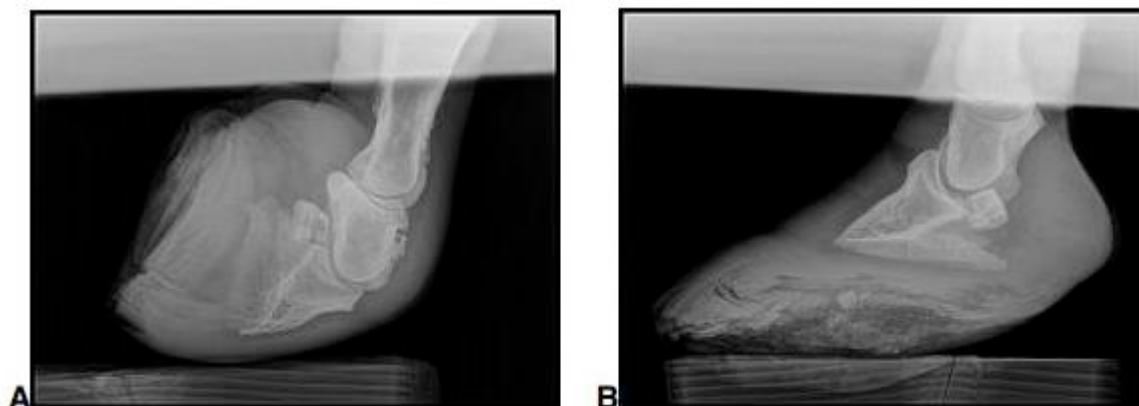
The echographic examination revealed no changes of the tendon fibers, both with respect to the area of cross section of tendons and ligaments examined. There were no pathological changes in any of the cuts (longitudinal and transverse) conducted along the said examination.

**Figure 23.** Angle between the wall and the soil on the wall foals affected.



**Figure 24.** A and B. Picture Radiological facilities CVM. The image on the right forelimb FD presents at an advanced stage, it is not possible to support the sole of the hoof on the ground. It also presents the sub-luxation of the distal interphalangeal joint, bone remodeling and deformation of the distal phalanx and the proximal phalanx.

The second dorsal phalanx presents a cyst. In B, the left forelimb hoof sole presenting excessively thick due to lack of wear resulting from difficulties in moving this horse and subluxation of the distal interphalangeal joint.



### 3.5. Measurement of Cortical Bone

Measurement of cortical bone by ultrasound is a quantitative non-invasive method for determining the surface properties of cortical bone, the results obtained can be found in Table 2.

**Table 2.** Values of the speed of sound (VDS, m / s) obtained in several measurements MCIII

Date	Thoracic M.	Isabel	Palomina	Castanha	Fundi	Espartaco	Engenheiro	Gcastanha	Gpalomina	Gcastanhall
January	Right Dorsal	4188	3960	4016	3908					
February	Lateral Right	4228	4401	4031	3980					
March	Left Dorsal	4033	4118	4292	4219					
April	Lateral Left	4322	4425	4366	4157					
May	Right Dorsal	3966	4003	3994	3954	3870	4103	3931	3710	
June	Lateral Right	4220	4258	4223	4370	4020	4443	3891	3729	
July	Left Dorsal	4107	4117	4105	3960	3697	4138	3786	3818	
August	Lateral Left	4264	4132	4285	4279	3995	4329	4033	3673	
September	Right Dorsal	3861	3797	4013		3999	3888	3966	3952	4145
October	Lateral Right	4158	3997	4262		4279	4159	4089	4085	4088
November	Left Dorsal	4009	3705	4442		4177	3847	3975	3997	3886
December	Lateral Left	4279	4076	3918		4005	4187	4178	4217	4170

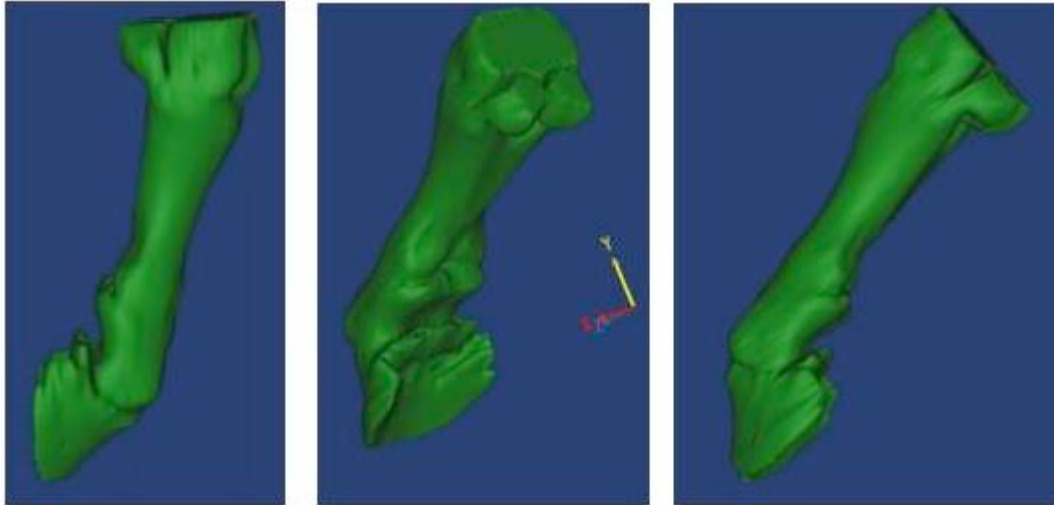
**Table 3.** Arithmetic mean of the speed of sound (VDS, m / s) obtained in several measurements MCIII the dorsal and lateral aspects measured in three different age groups.

Date of Birth	2009	2010	2011
Dorsal aspect	3965	4029	3933
Lateral aspect	4177	4203	4034

### 3.6. Computerized Axial Tomography (CAT)

The post mortem examination at the premises of FMV, confirms the marked flexural deformation of the distal interphalangeal joint (Figure 25).

**Figure 25.** Three-dimensional model of the program conducted by Mimics, using the front alignment of the three phalanges.



### 3.7. Desmotomy and biopsies of the accessory ligament of the deep digital flexor tendon

Desmotomy of the accessory ligament was performed as described previously.

Both surgeries were conducted in accordance with the provisions and the animals regained their proper conformation of the members soon after their recovery from surgery and remained in the field, (as it is a mare that would be used as a breeder). There was a recurrence of the FD, not as expressive as the initial, a few months later.

This was recovered with no incidence of recurrence following desmotomy. This filly was purchased by the owner of the stud from another Lusitano breeder having been obtained as a control animal. It was living in the pasture with the other homebred animals.

After nine months, it was possible to diagnose a change of conformation of the wall showing the beginning of the FD in this filly. This was carried out under sedation and local anesthesia of the animals. However, as mentioned earlier in this chapter, these two horses were placed in a field different from the one where they developed symptoms and showed significant improvements in the degree of FD clinically observed.



Collection of various tissue samples and the results of histological analysis are described in section below.

**Table 4** - Results of the horses in the study group.

Name	Year of Birth	Procedure	Results	Notes
<b>Canela</b>	2008	Desmotomy of LATFDP	Improvements after surgery	Remained in pasture. Relapse.
<b>Desplante</b>	2009	Desmotomy of LATFDP	Improvements after surgery	Recovery and permanence in stable. No relapse.
<b>Dondoca</b>	2010	Biopsy	Control animal. Developed FD of minor degree	Permanence in studs pasture.
<b>Engenheiro</b>	2010	Biopsy	Presented improvements related to FD	Permanence in studs pasture.
<b>Espartaco</b>	2010	Biopsy	Presented improvements related to FD	Permanence in studs pasture.
<b>Faneca</b>	2011	Necropsy		
<b>Fundi</b>	2011	Stabled at CVM	Presented improvements related to FD	Permanence at CVM.
<b>Fadista</b>	2011	None	With FD	Permanence in studs pasture.
<b>Formosa</b>	2011	None	With FD	Permanence in studs pasture.
<b>Furacao</b>	2011	None		Suffered accident necessitating euthanasia
<b>Flamengo</b>	2011	None	With FD	Permanence in studs pasture.

### 3.8. Analyzes anatomohistopathological

Was conducted by the Department of Pathology, Faculty of Veterinary Medicine of the necropsy of a horse – “Faneca”, November 10<sup>th</sup>, 2010. Regarding the gross lesions beyond FD evident in both the forelimbs, there is some irregularity in the floor of the oral cavity in the base of the tongue; lumps of 2 to 3 mm laryngeal mucosa, slight congestion and laryngeal cartilage and a bloody liquid in the pericardial sac (about 5ml).

Regarding the microscopic lesions observed hypertrophy and vacuolization of cells in the fascicular region of the adrenal cortex patents being dispersed some cases only vacuole formation. Zone cells of the outermost layer fascicular appeared slightly atrophied, with mild breakout. In the kidney was found occasional presence of hyaline and cellular casts in the lumen of the kidney tubes and hyperplasia of the epithelium lining the pelvis.

In the base of the tongue was found a luxuriant development, but within normal range, lymphoid tissue. The intracellular fibrils in the myocardium tissue cells cardio-nector appeared scattered throughout the cytoplasm, with no significant change in muscle fibers

At the level of the lung was found mucus in large quantities in the lumen of the large bronchi, sometimes filling it completely. The small bronchi appeared collapsed, also containing mucinous material in the lumen. The PAS staining for mucopolysaccharides revealed irregularities in the distribution of goblet cells in bronchioles virtually devoid of these elements. The interstitial tissue surrounding the vessels showed disorganized collagen fibers appeared separated by hyaline matrix. Van Gieson staining for collagen confirmed some disorganization of adventitious vessels of smaller calibre.

The alveoli showed moderately collapsed without content. In the tracheal mucosa were observed goblet cells are very large and sparse.

The oesophagus showed a possible rupture in the muscular wall with hemorrhage and oedema. The stomach revealed no structural changes worthy of record, and the mucosa appeared covered with material rich in mucins.

We observed absence of glands and fibrous tissue thickening of the endometrium in the uterus.

Ovaries observed developing follicles, containing fine granular layer. Throughout the stroma was invaded by macrophages containing pigment choroid, which was confirmed with Sudan Black staining.

The duodenum showed mild inflammatory inflammation of the lamina propria, consisting of mononuclear cells, considered within the normal range. Identified discreet oedema of the outer muscle layer.

Jejunum was found intracellular oedema of the muscular wall outside, the severity of which was not homogeneous across its border. The inner layer also appeared altered although more discreet, with distortion of cell layout. The mucosa showed the same pattern of infiltration as observed in the duodenum.

In the colon, the outer muscle layer appeared thinner than usual, with cell atrophy and intercellular oedema. Identified mucosal infiltration by eosinophils.

The mesenteric lymph nodes also showed scattered infiltration by eosinophils. The exuberance of the lymphoid tissue was subject to registration but may be due to the young age of the horse.

There was interfibrillar oedema, although discrete, of the fibrous trabeculae of the spleen.

In the liver, the hepatocyte cytoplasm appeared granular, with occasional presence of intracellular vacuoles small.

The thyroid showed follicles full of colloid in the two glands.

The pancreas, the parotid and pituitary revealed no significant changes.

In the bladder we identified intracellular and intercellular oedema longitudinal bundles of muscle itself, also evident in the form of small vacuoles transversely cut the beams.

In the brain, there were pictures of satellitism around several cortical neurons. In some areas, we identified numerous hypertrophied astrocytes. Neurons in the basal ganglia was also identified pictures satellitism. Regarding the cerebellum we identified occasionally cells Purkinje of angular outline and eosinophilic mild hypomyelination and white matter.

The brain stem showed dilatation of myelinated fibers, suggesting loss of myelin in the peripheral beams.

The pharynx (including post-mouth) and larynx showed an exuberant development of subepithelial lymphoid follicles, which may correspond to a situation of normality, especially in animals of this age group. The glottis showed the same type of subepithelial follicles.

For the striated muscle of the cuts made, particularly the longitudinal exhibited dissociation of myofibrils, although maintenance of transverse striation. Cross-sectional dissociation was also evident.

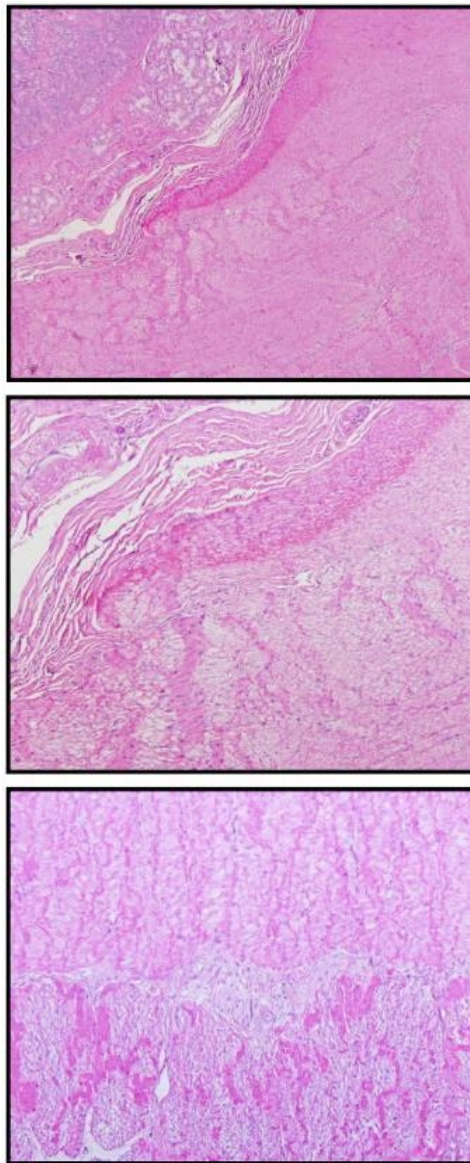
In relation to the tendons and ligaments in the proximal portion of the flexor tendons of the metacarpal region, the tendon tissue was morphologically consistent with the normal situation. However, the change was visible in the wall of some vessels, which resulted in abnormal thickening of the intima and subintima, coming to check on the almost total blockage of the lumen, or apparent dissociation of muscle fibers average, among which have collagen fibers.

The soft tissues surrounding the tendons showed intense vascularization by small vessels capillariformes sometimes arranged in continuous rosary. The structure of the deep flexor appeared particularly well preserved, however, one of the vessels of the periphery, with profile artery presents decoupling fiber average. In the middle area of the carpal ligaments and tendons did not identify significant changes in the tissue constituents.

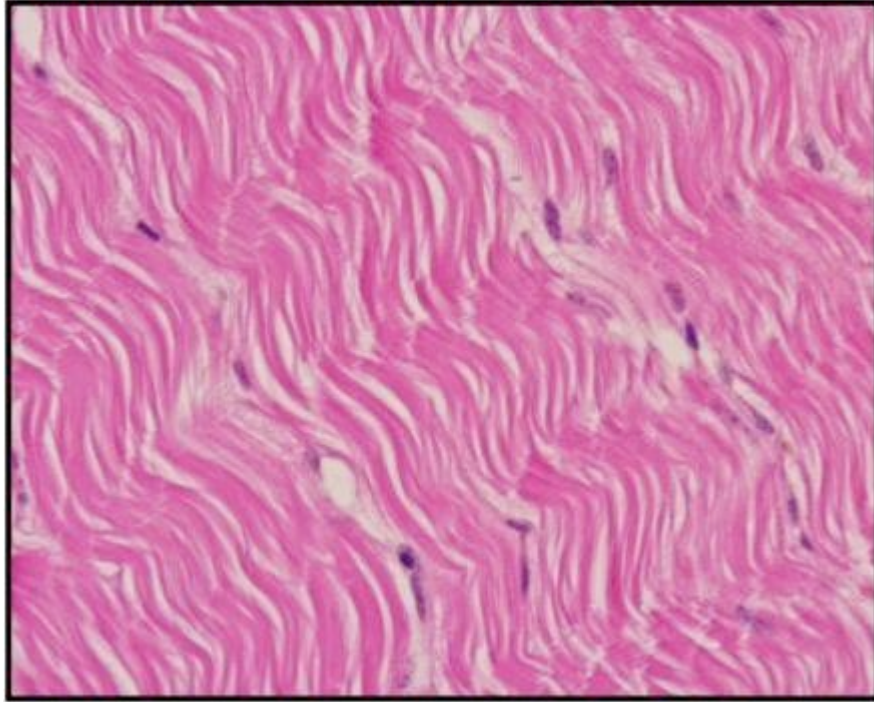
However, the presented vein scarcity of muscle fibers in its wall. In the superficial flexor tendon no significant changes were observed. In the distal tendons and ligaments no significant injuries were observed. Here also it was also possible to identify vein wall apparently depleted in muscle fibers.

Regarding the tissue samples collected from the two equine foals tendons in addition to sources of bleeding in the surrounding soft tissue, there were no changes compatible with the pathology course tendon.

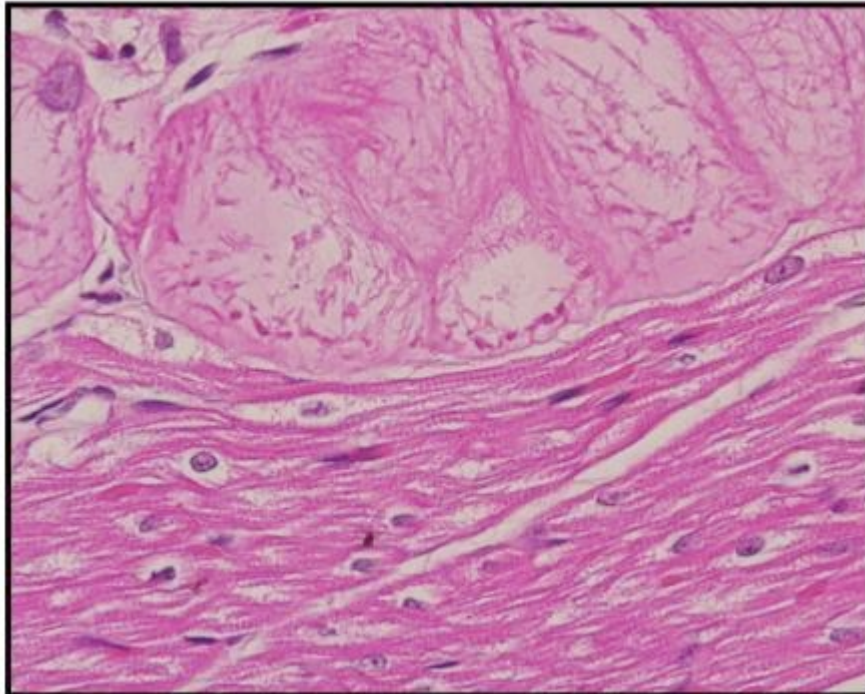
**Figure 26.** All photographs show intracellular edema and dissociation of smooth muscle cells of the intestinal wall. H & E, x40



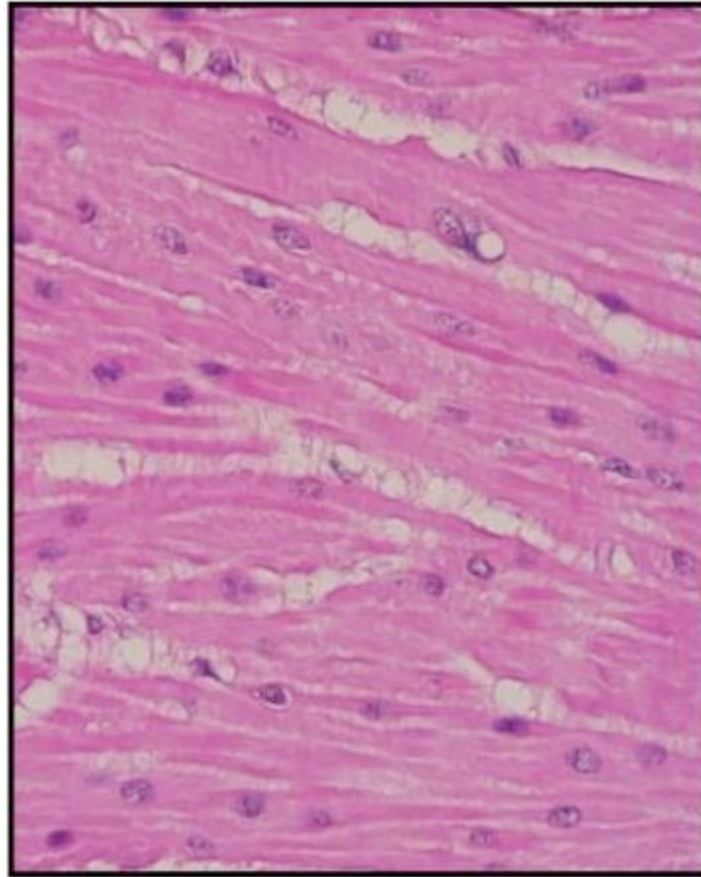
**Figure 27.** Dissociation of skeletal muscle fibers. H & E, 400x



**Figure 28.** Fabric cardionector presenting fibers randomly distributed. H & E, 400x

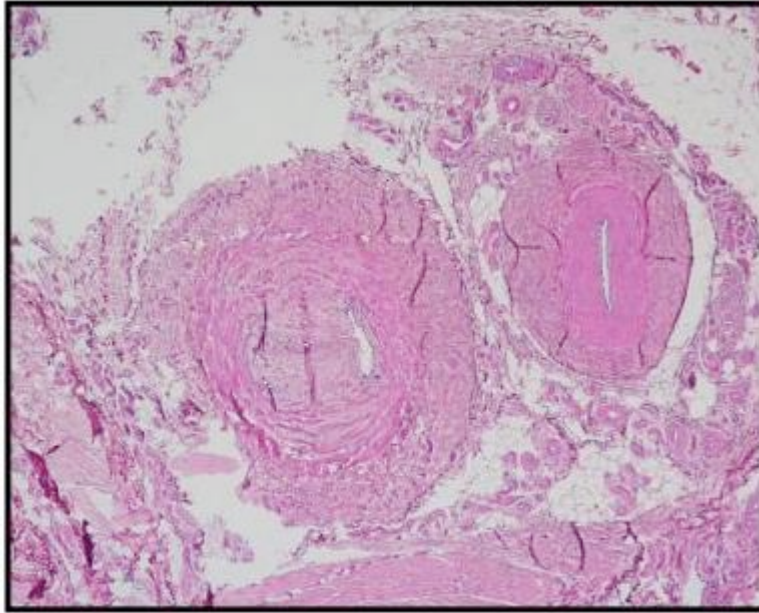


**Figure 29.** Intercellular edema of smooth muscle fibers of the bladder.

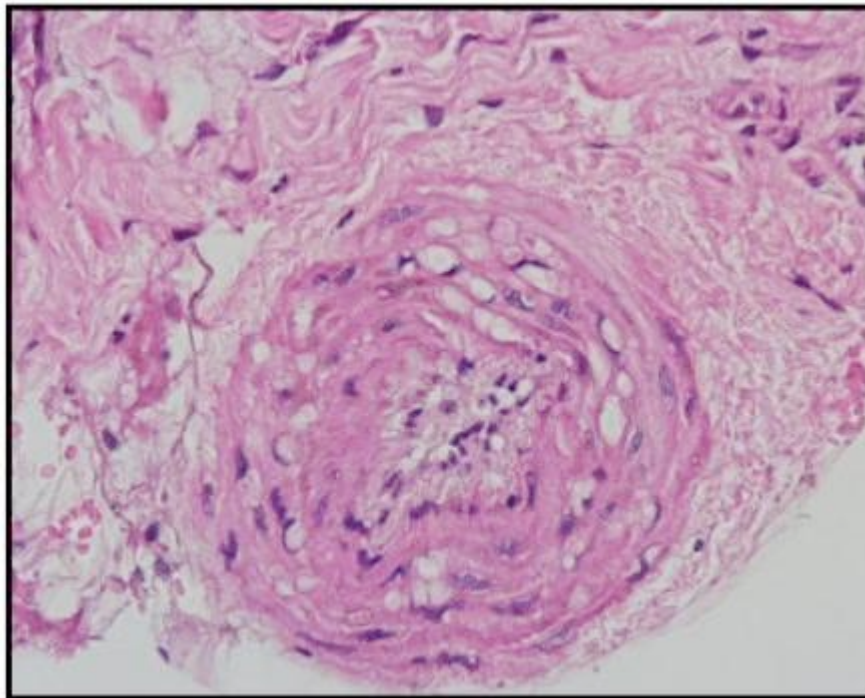


**Figure 30.** Vessels of the tissue adjacent to the superficial digital flexor tendon surrounding a blocked artery, left. The structure of the tunica media shows cell dissociation and fibers. H & E, x40

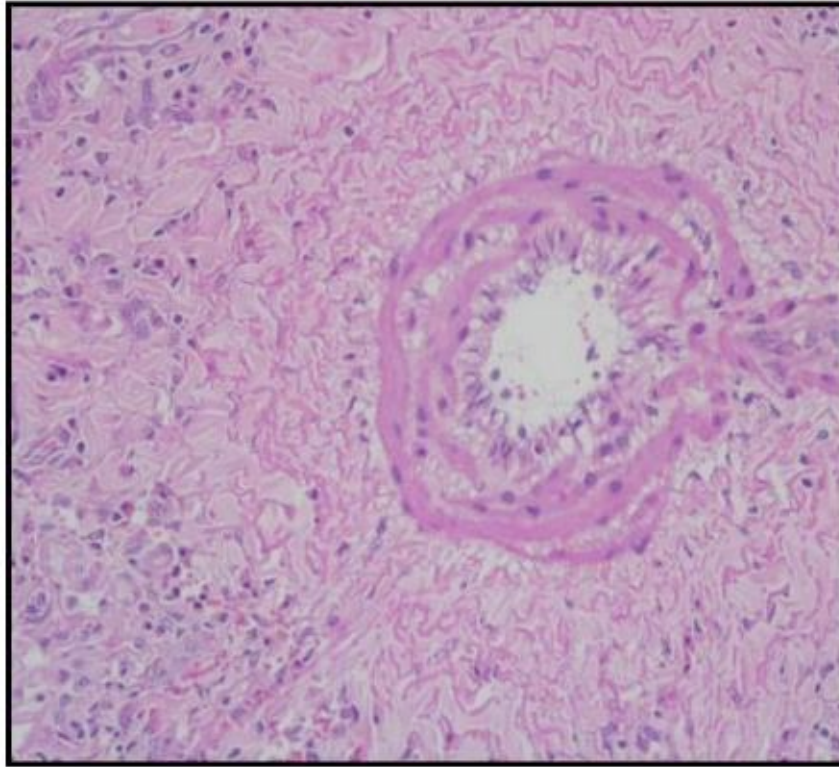




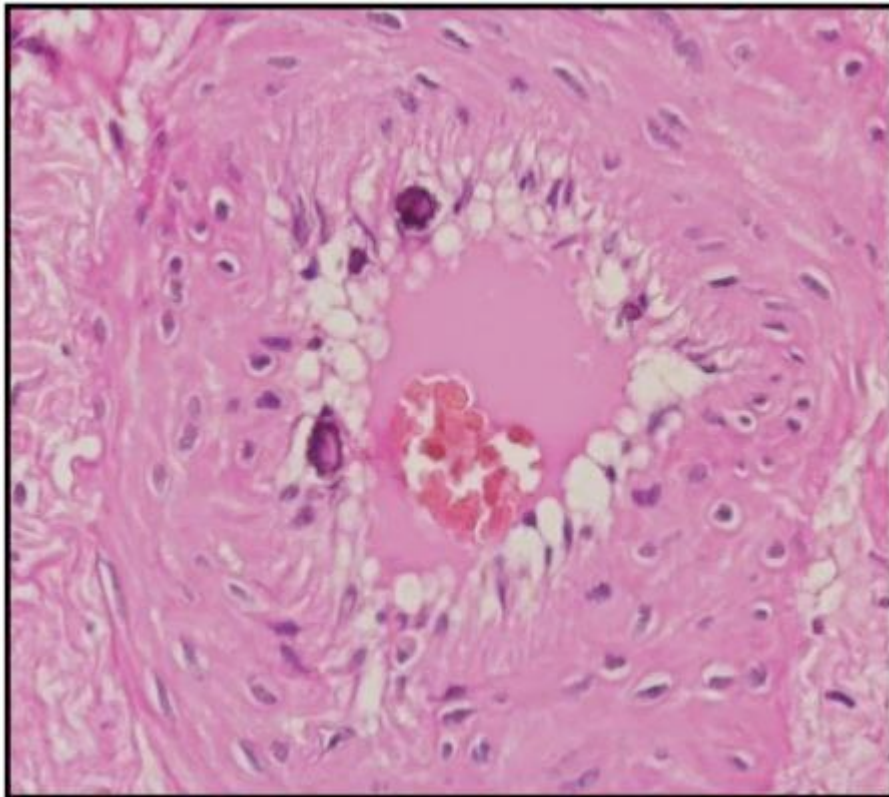
**Figure 31.** Vessels of the tissue adjacent to the superficial digital flexor tendon.  
Edema in the tunica media cells (H & E x400).



**Figure 32.** Small artery in the lungs, showing dissociation of the cells of the tunica media and adventitia.  
(H & E x400)



**Figure 33.** Small artery in the lungs, showing subintimal corpora intimal calcification H & E x400





### **3.9. Noise measurements**

Quantification noise measurements were made at various locations on the stud and very close to the turbine itself.

The noise measurements carried out, captured low frequency noise at different frequencies and tonal noise (noise in the frequency emitted always shows the same value), mainly from the gears of the wind turbine rotor. Measurement campaigns were carried out over several days and at different times of day and weather variables. Nevertheless, measurements of noise and its propagation, require a detailed processing of the data as well as expert analysis to produce comparable facts which will enable the establishment of a possible correlation between them and the changes that have manifested in the equine Stud.

During this study it was not possible to infer safely on the relationship between the frequencies of the sound waves emitted by the TE and cellular changes described in the point above or injuries that may result from this same noise disturbance with scientific reliability.

### **3.10. Vibration measurements of the soil**

In Annex (Annex I) are full results and report conducted by ICIST / IST (Institute of Structural Engineering, Territory and Construction, IST)

Studies have been conducted measuring mechanical vibrations. These were performed by ICIST / IST (Technical University of Lisbon) at the request of the Faculty of Veterinary Medicine.

The vibrations induced by the operation of the wind towers (moving blades and other existing mechanical equipment) have a frequency content concentrated in bands with medium frequency 3 Hz, 8 Hz, 15 Hz, 28 Hz, and especially around 55 Hz. It is believed that some of the bands may vary with the speed of movement of the blades (which in turn is correlated with the speed of the wind).

The vibrations existing within a holding concentrated in the frequency band next, lower, at 30 Hz and above (again), close to 60 Hz

Possibly the concentration around 30 Hz manifests itself mainly in the radial components (whose transmission involves compression waves in the soil).

The analysis of the previous results seem to point to the fact that the vibration induced by the operation of the wind towers to attenuate very rapidly with distance from the source (comparing records obtained in E with those from A1 to A3).

Within the scope of this attenuation operation is already quite low, such that the zone closest to the wind tower there is a direct correlation with distance and unambiguous the same towers (comparing logs obtained in A1, A2 and A3).

The attenuation with distance is observed in a much more mitigated to more distant areas of the holding areas and to have considerably distant from the operation (comparing logs obtained in A1, A2 and A3 with those obtained in D and F also).

It should be noted that there has been no analysis of the influence of the intensity of the wind-induced vibrations in, since this intensity was comparable in the two campaigns of measurements.

The analyses performed in the frequency domain point to a strong attenuation of the movements induced at lower frequencies, below 30 Hz, although there remain very attenuated frequency ranges of 30 Hz and near 50 Hz

#### **IV – DISCUSSION**

In the eleven horses in the study group we confirmed diagnosis FDAAID, by performing clinical exams, orthopedic, radiographic, ultrasound and goniometry.

The query references available does not register as high prevalence of this disease in only one stud and a small number of animals.

The FDAA ID diagnosed were evaluated and quantified featured within the clinical and orthopedic performed. All animals found to the emergence of FD forelimbs. These support, higher body weight (60% by weight of the horse) that the hindlimbs, and that the latter, the angle of the joints allows greater adjustment and monitoring of muscle-tendon tissue in relation to bone growth.

6 horses found in the expression unilateral deformation. This pathology studied with rapid development. As reported, investigated the clinical histories and records of stud orthopedic injuries, as well as fewer young horses that live there and were born before the change of environmental conditions. Regarding the history of lesions observed in the stud, it reports that in the years prior to the introduction of the TE in the neighboring land, there were no forum orthopedic injuries similar to those described in this work. All horses born before 2008 were observed and the stud properly considered neat, i.e. not exhibit FD.

With regard to diagnostic exams, radiological findings were compatible with the FD observed clinically. There was marked shift in the axis of the phalanges in the sagittal plane, subluxation of the distal interphalangeal joint and bone remodeling at the distal and proximal phalanges, due to forces that are subjected to pathological and caused by the changes undergone by the lack of support of the sole Wall the soil.

Sonographically, as expected, were not visualized changes of tendinous fibers, whether the morphological level both within the fiber structure. As indicated in the literature, FD develop pathology or alteration without tendon.

There is only an abnormal rate of growth between the bone and the structures of the limb flexor.

During the clinical examinations performed to horses in the study group were not identified pathological processes that could lead to pain at the distal end, such as abscesses or haematomas sub-solar epiphysitis and other processes already described in Chapter I of this work. Therefore, we excluded the pathogenesis of painful causes of FD, that could lead to the contraction of the muscle-tendon and consequently the said FD.

With regard to results in the realization of quantitative ultrasound collected data were compared to data obtained and published by Fradinho et. al. 2009.

According to the author, the measurement values of VDS on the lateral aspect of the MCIII increased significantly with age, while the same was not observed in measurements made on the dorsal aspect of said bone. Values were similar for the horses in the study group and the other foals Lusitano stud farms in Portugal.

One aspect of importance in this study was the fact that two foals studied were not born in the stud farm and therefore they have no connection with any other equine genetic group and yet, after only a few months on the pasture, developed the FD. These two animals were obtained from a phase of development in which bone growth occurs much faster.

In the equine species, the end of the first 12 months, on average colts present, approximately 80% of the dimensions that they will have as adults.

Note that in all animals in the study group, the FD occurred at a very young age, hence the phase of rapid bone growth.

Considerable aspect was the observation of clinical improvement in three horses of the group when removed from pasture early, unlike the three other young animals, born in 2011, who remained in the pasture and continue to display the FD.

It is noteworthy also, that the animal which remained stabled following recovery from the LATFDP did not evidence a relapse/recurrence whilst those returned to pasture after initial recovery from surgery, came to redevelop FD.

As mentioned earlier in this paper, cells in general and of particular interest to this study, the cells of tendons and bones, respond to mechanical stimuli.

This response is expressed in and structural changes even in the composition of said cells. This mechanism, described in Chapter I of this paper, is called mechanotransduction.

In that case, the actin cytoskeleton is present in a decisive role, as it transmits and modulates the voltage across the ECM, the local focal adhesion and integrin conformational changes leading to the latter, G-protein and the composition of channels ion.

These conformational changes, stimulate membrane receptors and induce complex biochemical cascades (including activation of transcription factors) with subsequent protein synthesis, gene expression and cell differentiation. However, the histology performed, the tendinous tissues evaluated did not show significant changes in their structure, while the level of other tissues of various organs, tissue damage apparently been recorded material.

Having ruled out the influence of feeding, management and genetic origin contributing to the appearance of unusually frequent cases of FD in the stud in study, it remains to evaluate the influence they may have had environmental changes. However, the only change that has occurred from the time the disease started to check consistently been assembly of the seven turbines on the ground in a semicircular arrangement, involving in particular the pasture where the animals graze, from birth until weaning. As mentioned in the introduction, there are two effects to consider in persistent activity of TE, there are minimal recorded pause periods at full operation. Occasionally one may be shut down for maintenance, but others remain always active, day and night. Thus, the noise is both continuous, increasing with decreasing wind speed (noise of the moving blades and rotor) as the vibration transmitted to the ground, being registrable only by high sensitivity seismographs, it is noticeable cells bone through the aforementioned process mechanotransduction.

According to research, there are studies that report the osteogenic growth induced vibration (Rubin et al., 2001).

In fact, the mechanism of mechanotransduction may have played a role in the process by which mechanical forces, vibrations in this case, the cells were subjected, may have led to a structural response of bone tissue growth. Consequently, the bone may have suffered exceptional anabolic stimulus, which was reflected in faster growth than expected.

Thus, the constant and continuous mechanical stimulus at the grazing of the stud foals as a result of installation of wind towers may have led to more rapid development of the bone, which was not accompanied by tendons, leading to DF acquired joint distal interphalangeal.

Regarding the tissue damage observed in the studied tissues, it is interesting to compare them with what is referred to in the literature as a consequence of the pressure of sound waves of noise, including noise and low frequency infrasound can cause the cells (Alves Pereira and Castelo Branco, 2002).

It is disclosed in some publications that when the frequency emitted by the source of noise and vibration is similar to the natural frequency of a particular cell or tissue can lead to the occurrence of the resonance phenomenon. This physical phenomenon acting in a specific manner for each frequency, with a certain type of cell can be stimulated over another, depending on the different frequency that is exposed.

However, measurement noise and its propagation requires collection and data processing thorough and complete conclusions to achieve scientific interest in order to be able to establish a correlation between the frequency of these sound waves and the natural frequencies of the injury and animal cells that may arise from this same noise disturbance.

In the present study no reliably scientific data were found about the natural frequencies of the cells of horses that could safely infer about the effect of noise in the immediate vicinity of the stud with the cellular changes of these animals.

In the literature, there are few references about natural frequencies of animal cells in comparison to studies performed in humans that are already set values for the different cells, organs (Hakansson et al. 1994).

Investigation and characterization of frequency of the sound waves that lead to cell injury may be a project specific scientific interest because it could lead to an understanding of disease whose etiology has not yet been fully characterized.

Regarding the phenomenon of vibrations propagated through the ground, it is worth noting an interesting study in Scotland, near the seismic station of Eskdalemuir (ESK). This station monitors possible explosions and nuclear activities, relying for such extremely precise quantification of ground vibration waves made by seismographs. The importance of measurements at this station for the maintenance of public security interests is British military led, after measurement campaigns carried out in various parks, was advised that no TE was located within 50km of the surrounding ESK. (Styles P. et al, 2011).

As mentioned in previous chapters of this work, the stud in study were also conducted measurement campaigns of mechanical waves propagated in soil for characterization of these vibrations. Understanding the pathway that leads to this DF is important in that only in this way can take preventative measures to prevent its occurrence and avoid

other possible biological effects that may be harmful and thus find solutions to reduce or avoid.

In 2005, there was no technological solution to mitigate the vibration emitted by TE that could lead to its reduction. At present, systems have been developed which can be effective in reducing the vibration transmitted to the ground, for example technology developed by the damping can be adjusted or installed at the time of construction of WT.

This technology was tested by styels et al, at the University of Keeley in 2009, in the context of the study referenced above, commissioned by the Ministry of Defence, Economy and Trade of the United Kingdom and the institution that regulates renewable energy in this country (UK Renewable)

The vibration mitigation technology developed and used in the TE region ESK to ensure the reliability of seismic measurements, has high potential to be applied in wind farms which generate TE frequencies and / or amplitudes that can be problematic for neighboring regions.

## **V – CONCLUSION**

The present study aimed at studying the acquired flexural deformities of the distal interphalangeal joint (DFAAID) foals on a stud farm which, in previous years, showed variations in their environmental conditions due to the installation of wind towers (ET) on adjacent land.

The understanding of the pathogenesis of this condition would determine flexural to infer about the possible causes underlying the high incidence of DF observed in stud.

Once in the course of successive examinations to equine group, we excluded the existence of pain in primary forelimb that could lead to the shortening of the muscle-tendon of PRFD and the resulting DF, also considering that the pathogenesis of the process can be due to bone growth faster than the development of the tendon, the latter seems to be associated with the pathogenesis DFAAID observed in this group of animals.

Given the mechanical forces produced by TE deployed on land adjacent to the stud, and we can not definitively conclude as to its responsibility in the process, one can not, however, rule out the influence of the low frequency noise and mechanical vibration may have had in the pathogenesis of DF verified.

For proof, it would be necessary to have means that are outside the scope of this thesis. These means would require significant financial resources that allow creating an

experimental environment in fully controlled conditions of vibration of air and soil that foals are subject to when they are born, by monitoring the changes of bone growth.

The fact that, in foal necropsied at seven months, there were several structural changes in tissues that contain cells rich in fibrillar skeleton (cardionector fabric, smooth and striated muscle), suggests that this issue of the influence of vibrations on cell growth should be targeted for further studies.

The TE bit is seen as harmful to the environment, with the Portuguese legislation only required environmental impact studies when the same park contains more than eight towers.

However, should it prove in the future that their presence may have harmful effects, there will be more careful in preventing its installation in the vicinity of population or livestock.

In ignorance of these effects, and now under suspicion, it already seems sufficient reason for precautions are taken as to where the future will be authorized its installation.

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