The effect of loading upon hoof wall growth and hoof shape in the Thoroughbred foal

by

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Declaration

Concurrent registration for two or more academic awards:
I declare that while registered as a candidate for the research degree, I have not been a registered candidate or enrolled student for another award of the University or other academic or professional institution.

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Abstract

The hoof wall is adapted to take most of the weight-bearing of the foot and is anisotropic and homogeneous. Foals appear to be born with symmetrical paired feet which by maturity are frequently unequal in angle and width. They stand within minutes of birth subjecting the hoof wall to loading. Hoof growth rate and hoof compression may be factors affecting hoof shape. The effect of conformation changes during maturation upon loading and differential hoof growth was unknown. The aims were to; quantify and evaluate the epidermal structure, hoof growth rate, hoof renewal, dorsal hoof wall angle, plastic hoof compression, and hoof loading, during paediatric development.

Hoof growth rate, renewal, and hoof angle were recorded in foals (n=80) and weanlings/yearlings (n=12) and the hoof wall structure of histological samples of fetuses and paediatric foals (n=15) was determined. Solar loading, hoof growth, and hoof angle was recorded in healthy foals (n=18) and compared to a group with acquired flexural deformity (n=9).

Horn tubule size and number increased significantly, and density decreased significantly during maturation. The dorsal hoof wall angle declined with age. Hoof renewal in newborn foals was 145±15 days whereas weaning/yearlings were 283±26 days. A relationship between plastic hoof compression and time was found (r = 0.46, P = 0.002). Hoof compression in foals was 0.039±0.022mm per day and in weanling/yearlings 0.03±0.016mm per day. Hoof growth rate, hoof compression, and dorsal hoof wall angle, all correlated to ageing and differed between the healthy and acquired flexural deformity foals.

The original findings in this thesis give a broad understanding of the developing equine hoof wall. Knowledge of renewal times allows predictions of healing in cases of partial hoof wall avulsion. Original data recording the structure of the developing hoof wall may lead to a greater understanding of its response to loading, while improved recognition of the angles of the digit and hoof during maturation will allow a more accurate assessment of conformation. Hoof distortion is affected by loading, hoof compression and hoof growth and comprehending the link between these factors may lead to enhanced treatment strategies for all ages of horse.

Table of Contents

Illustrations and tables

List of illustrations

List of tables

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My special thanks go to my wife, Beverley, without whose continual encouragement and backing I could neither have undertaken nor completed this thesis.

Excellence is an art won by training and habituation. We do not act rightly because we have virtue or excellence, but we rather have those because we have acted rightly. We are what we repeatedly do. Excellence, then, is not an act but a habit.

In all things of nature there is something of the marvellous.

Aristotle 384–322 BCE

Abbreviations and definitions

Abbreviations

AFDdipj; *acquired flexural deformity of the distal interphalangeal joint*

AFD; *acquired flexural deformity*

CH; *coronary hairline*

COP; *centre of pressure*

C%; *compression percentage*

DDFT: *deep digital flexor tendon*

DHWA; *dorsal hoof wall angle*

DID; *distal integument depth*

DP; *dorsopalmar*

DPA; *dorsal parietal angle*

FHC; *foal hoof crease*

HCR; *hoof compression rate*

Hz; *hertz, one cycle per second*

HGR; *hoof growth rate*

HPA; *hoof pastern axis*

KJ; *kilojoule*

Kg; *kilogramme*

LF; *left fore.*

LM; *lateromedial*

mm; *millimetre*

MSC; *midline sagittal centre*

N; *newton, unit of force*

PCA; *principal component analysis*

PEL; primary epidermal lamellae

PID; *proximal integument depth*

PIII; *distal phalanx*

RF; *right fore*

RH; *reference hairline*

SBA; *solar border angle*

SDFT: *superficial digital flexor tendon*

TB; *Thoroughbred*

µm; *micrometre, commonly known as a micron*

Ur; *unmeasured hoof remnant*

Definitions:

Acquired flexural deformity (AFD); *abnormal flexion of the limb along a dorsopalmar plane.*

Acquired flexural deformity of the distal interphalangeal joint (AFDdipj); *a flexural deformity specifically affecting the coffin joint and foot.*

Axial hoof wall; *the stratum medium towards and adjacent to the stratum. internum* (Figure i.1).

Abaxial hoof wall; *the stratum medium towards and adjacent to the stratum externum* (Figure i.1).

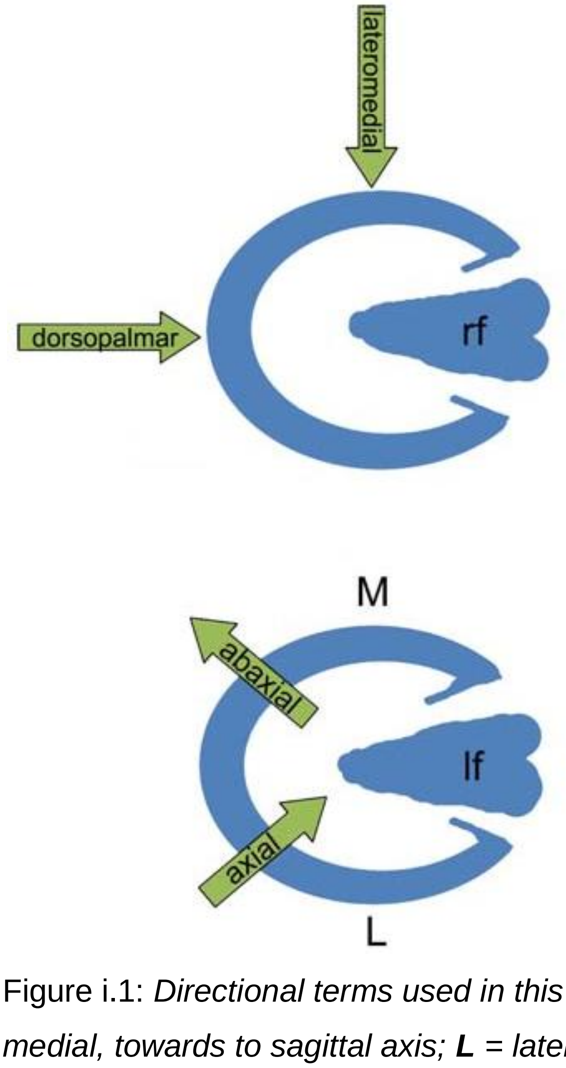


Figure i.1: *Directional terms used in this thesis;* ***lf*** *= left fore;* ***rf*** *= right fore;* ***M*** *= medial, towards to sagittal axis;* ***L*** *= lateral, away from the sagittal axis;* ***axial****, towards the centre of the foot;* ***abaxial****, away from the centre of the foot;* ***dorsopalmar****; a view from front to back;* ***lateromedial****, a view from lateral to medial.*

Break-over; *the part of the stance phase when the heels leave the ground and the hoof capsule rotates forward, until the toe leaves the ground.*
Club foot*; a condition of the front foot with a number of characteristics differing from a typical conformation, including; a broken forward (positive) hoof pastern axis (HPA), steep dorsal hoof wall angle (DHWA), high heels, separation of the dorsal white line, hind shaped solar outline* (Figure i.2).

Figure i.2: *The club foot (closest) has a number of characteristics different from the healthy foot (distant) including; a steeper dorsum, higher heels, growth rings divergent at the heel, a positive hoof pastern axis.*

Conformation; *the silhouette shape of the horse's body, more generally used to describe overall three dimensional body shape.*
Dorsopalmar (DP); *the direction of a radiographic beam or optical view from the front of the foot towards the back* (Figure i.1).
Foal; *a Thoroughbred horse aged from newborn to December 31 (Northern Hemisphere).*
Foot of the horse; *the hoof capsule and all that is contained within it.*
Hoof of the horse; *the horny capsule surrounding the tip of the digit* (Figure i.3).
Hoof wall; *the part of the hoof, seen when the horse is standing, which is generated from the coronary corium*.

Figure i.3: *The hoof wall is the part of the hoof capsule visible in a standing foot (lateral view).*

Hoof pastern axis (HPA); *the dorsal hoof wall angle (DHWA) minus the pastern angle; this can be straight, broken forward (positive), or broken back (negative).*
Lateral; *away from the centre (sagittal axis) of the horse* (Figure i.1).
Lateromedial (LM); *the direction of a radiographic or optical view from the lateral side.*
Histology: *the study of the microscopic structure of tissues.*
Medial; *towards the centre (sagittal axis) of the horse* (Figure i.1).
Paediatric foal; *aged from birth to approximately five months.*
Phalangeal axis; *the dorsal parietal angle (DPA) minus the mean of the combined angles of the proximal (PI) and middle (PII) phalanges.*
Thoroughbred (TB); *a breed of horse specifically developed for racing.*
Weanling: *a period in a TB foal's life, from when separated from the mare to December 31(Northern Hemisphere).*
Yearling*: a Thoroughbred horse in its second calendar year (Northern Hemisphere).*

In this thesis the anatomical terms used are taken from *Nomina Anatomica Veterinaria (2012, revised version).*

Chapter 1 Introduction

In this chapter the present literature is reviewed and an over-arching description is given of where this thesis sits in the understanding of the equine digit and hoof wall, especially with regard to the Thoroughbred foal. The evolution of the digital integument of the horse's foot is considered. The anatomy of the equine foot, with emphasis on the development and structure of the hoof wall, is described and illustrated and there is a brief explanation of the Thoroughbred industry.

1.1: Evolution

1.1.1: *Integument*

Integument is the organ covering the exterior of vertebrates, consisting of the dermis, epidermis and subcutaneous layers, which mediate between its organism and environment. In reptiles it is characterised by scales, in birds by skin and feathers and in mammals by skin and hair. In certain regions of the body it has specialised adaption such as avian beaks and mammalian lips (Bragulla and Hirschberg, 2003). Newly germinated living cells undergo cytodifferentiation and it is during this basic process that these modifications occur. Keratinisation occurs during the procession of cells away from the germinative layer (Pollitt, 1998).

1.1.2: *Adaptation of the digital integument*

At the extremities of the digits integument adaption is described as claw, nail, and hoof and is modified to each individual species depending on use, especially locomotory (Bragulla and Hirschberg, 2003). The form of the distal phalanx and the enveloping integument vary according to use. The unguis is squat in terrestrial, cursorial ungulates such as deer that travel on relatively level ground, whereas it is sharp, short and narrow in climbing mammals such as cats. The wide variations in digit and integument adaption at the tip shows that the evolutionary diversification has played an important role in the different feeding and foraging behaviours of terrestrial animals (Hamrick, 2001). In human beings the nail is located only at the dorsal tip of the digit, in ungulates, which bear weight on the extremity of the digit; it is further adapted to give protection, grip, and resistance to wear. The fossil remains of horse foot imprints, from the Pleistocene epoch, suggest an evolutionary trend toward thicker hoof walls in monodactyl equidae and at this time the frog structure first appeared (Reynolds, 2006).

1.1.3: *Evolutionary adaptation*

The genus equidae which includes; the donkey (*Equus africanus asinus*), three species of zebras (*Equus zebra hartmannae, Equus grevyi, Equus quagga*), and the horse (*Equus caballus*), has numerous evolutionary adaptations to the foot which enhance locomotor speed by grip, purchase and protection. Uniquely equidae have a single digit on each limb enclosed in relatively light-weight horn (Bragulla and Hirschberg, 2003). The hoof capsule, which encloses the tip of the digit apart from the ventral aspect, is divided into three major distinct horny areas and two minor. The hoof wall, sole, and frog constitute the majority of the hoof with each adapted to its specific functions. The periople (*stratum externum*) covering the junction of the skin and hoof wall and the white line (white zone) joining the hoof wall and sole, are considered minor hoof horn (Figure 1.1).

Thirty million years of evolution have selected an animal that stands immediately *post partum* on the tip of its third phalanx and directly follows its herd. By the evolutionary adaptation of a single digit the horse has become an animal of extraordinary athletic prowess. This has allowed it to inhabit large areas of the planet, and its use to humankind ensured its survival as a domesticated animal. It has been utilised as a beast of burden, able to carry and pull loads for agriculture and commerce. Ridden, it was for millennia, indispensable for human expansion and conquest; now for sport and leisure it is ubiquitous in the developed world. Intensive breeding has lead to further adaptation of the horse's physique including the hoof.

The variety of physical types, from stout draft horses to the fine elongated limbs of the Thoroughbred and short Shetland ponies, are reflected in similarly adapted hooves. Thoroughbred hoof integument is proportionately thinner and therefore lighter than other breeds and this one of many traits that has lead to this breed’s athletic primacy. However, less is understood of the ability of an individual horse's hooves to respond to different effects that impose upon them.

The effect of weight-bearing and loading upon the hoof wall is only partially evidenced. Changes in shape of the hoof capsule of the foal may be affected by development of the limb, conformational changes, the substrate, hoof wear, farriery and other factors. Some of these will be investigated in later chapters.

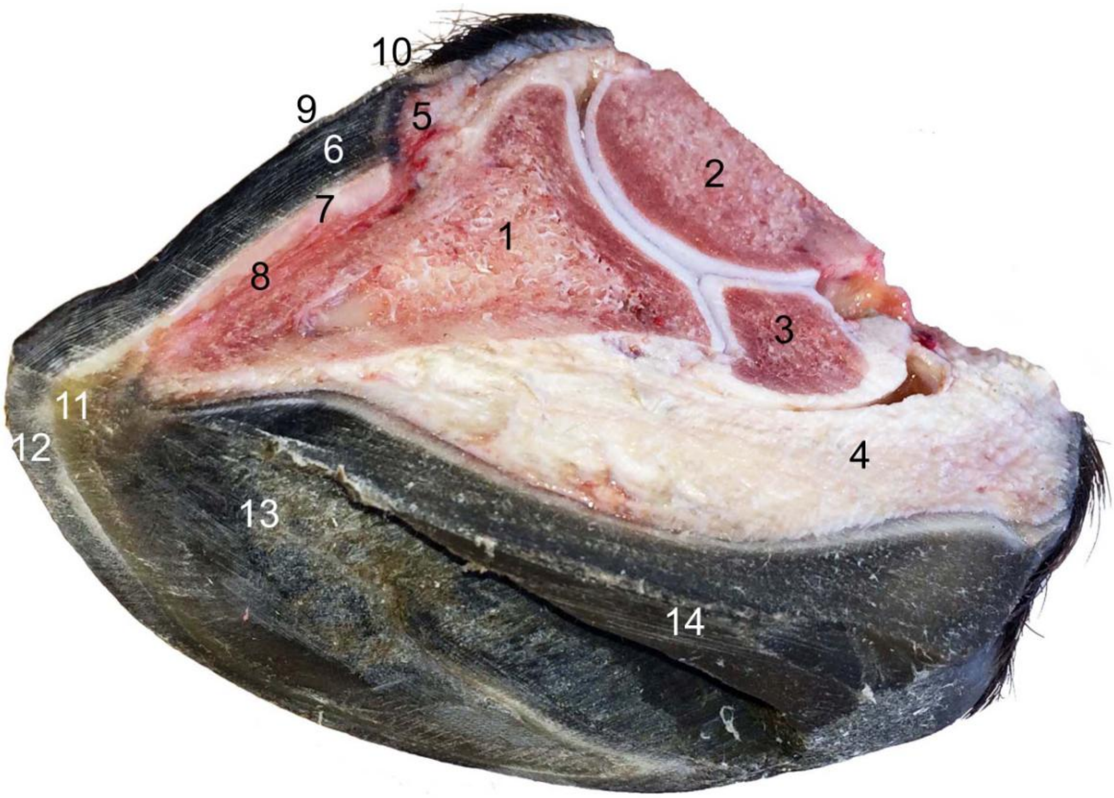


Figure 1.1: *An oblique view of the internal and external hoof capsule; 1) distal phalanx ; 2) middle phalanx (partial), 3) distal sesamoid (navicular bone); 4) palmar/plantar cushion; 5) coronary cushion, 6) pigmented hoof wall of the stratum medium, 7) un-pigmented hoof wall of the stratum medium or zona alba; 8) stratum internum; 9) stratum externum (periople); 10) coronary hairline; 11) white zone; 12) bearing border of the hoof wall; 13) sole; 14) frog.*

1.2: Anatomy of the hoof wall

1.2.1: *Hoof capsule shape*

The hoof capsule can be described as an oblique truncated cone where only the ventral surface is not horn (Kaspasi and Gosline, 1996). There are many variations and distortions to hoof shape and these may be caused by; wear, breed, conformation, injury, disease and farriery. The hoof wall is the part of the equine foot seen when standing and forms the largest part of the hoof capsule. The hoof wall has three discrete layers; the *stratum externum* (periople), the *stratum medium* (main body), and the *stratum internum* (connective region) (Figures 1.1 and 1.2).

1.2.2: *The stratum externum*

The stratum externum is generated from a narrow band of tissue located proximal to the junction between the hoof wall and skin. In life it can be distinguished from the hoof when wet where it becomes white. Its function appears to be to protect the junction between the skin and *stratum medium* and to control hydration of the *stratum medium*. It usually only extends distally for about one third of the proximal hoof wall and is eroded distally. The *stratum externum* seems to play no part in the structural integrity or biomechanics of the hoof wall (Leach, 1980).

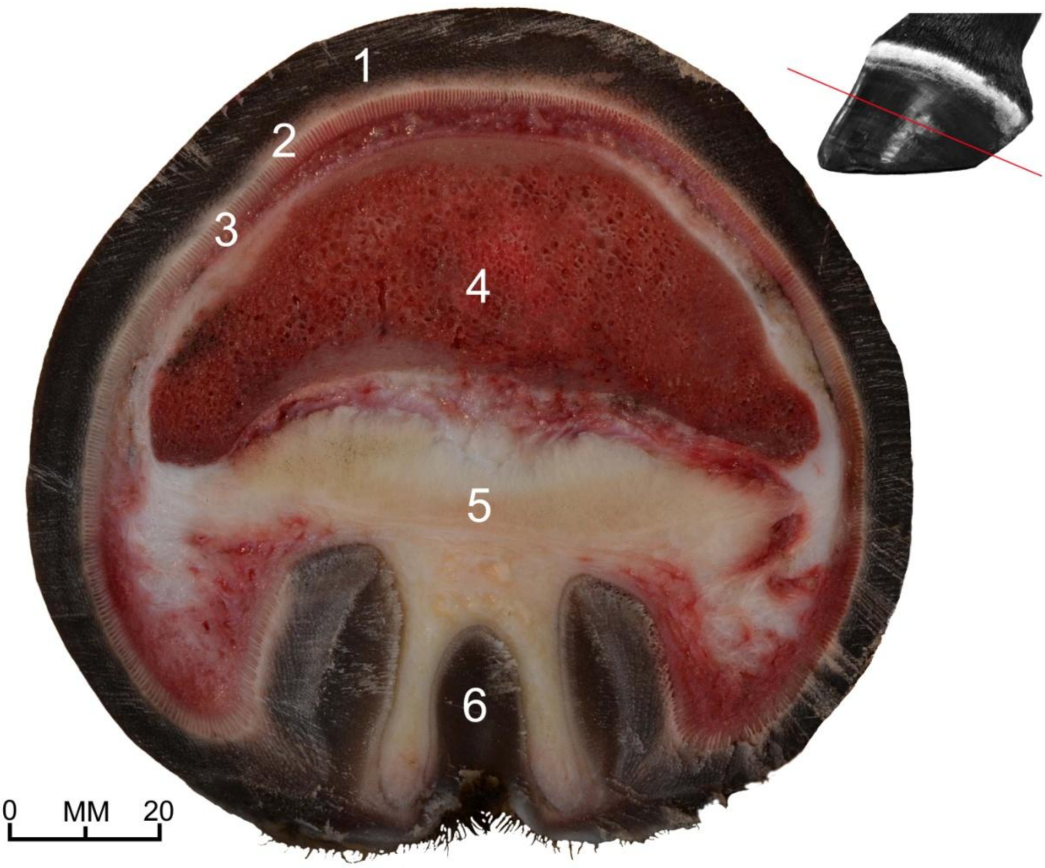


Figure 1.2*: A transverse section parallel to the coronary band through the equine foot; 1) stratum medium; 2) stratum internum; 3) stratum lamellum; 4) distal phalanx; 5) plantar cushion; 6) frog.*

1.2.3: *The stratum medium*

The stratum medium is a heterogeneous structure forming the main body of the hoof wall. It is generated from the coronary corium at its proximal extent, consisting of an epidermal basal layer and papillae which protrude distally invaginating the hoof wall. Germinal cells produce keratinocytes throughout the life of the horse which move distally away from the basal layer due to the continual generation of new cells proximally. Maturing keratinocytes either become organised into thin elongated horn tubules arranged around a hollow tube or mature into intertubular horn surrounding the tubules. The basal cells, whether destined to become intertubular or tubular cells, do not keratinise immediately but continue to form more desmosones which make strong attachments with adjacent cells. Within the cells keratin intermediate filaments also form attachments to the desmosones, creating the three-dimensional skeleton of the cell. The concluding phase of maturation is rapid, the cell expires and becomes anuclear and the cytoplasm is crammed with communicating keratin filaments which connect with other filaments and the desmosones (Pollitt, 1998). The continual layers of squamous keratinocytes become a stratified squamous epithelium. In this manner the *stratum medium* becomes the robust and durable material required to allow the hoof wall to fulfil its many functions.

1.2.4: *Tubular horn:*

The papillae, 4-5 millimetres (mm) in length, generate cells to form the cortex of horn tubules which move distally and progressively keratinise. These horn tubules descend, in a parallel relationship, to the distal (bearing) border of the hoof wall. Within the medulla of the horn tubules is intratubular horn generated from basal cells at the distal tips of the papillae (Figure 1.3). The tubular structure changes from internal (axial) to external (abaxial) in the *stratum medium*. The axial tubules appear to stiffen the wall along its longitudinal axis. Axially the tubules have six times the stiffness of the intertubular horn compared to mid-wall where they are similar (Kaspasi and Gosline, 1999). Many horn tubules have a round appearance when viewed in cross section; towards the abaxial hoof wall they are smaller with many more oval in cross section. These oval tubules are aligned through the long axis of the oval at a tangent to the outer wall (Pollitt, 1998). Leach (1980) illustrated three types of horn tubules:

inner type (Type 1), which occupied an area adjacent to the lamellae; second type (Type 2), which occupied an area of the mid-wall and were larger and round; and a third type (Type 3), which occupied an area adjacent to the abaxial surface and were small and oval (Figure 1.4). Between Type 2 and Type 3 is an area where horn tubules share the characteristics of both and Leach (1980) categorised these as Intermediate Type.

Figure 1.3: *A foal's foot with the hoof capsule removed;* ***a****) the coronary hairline,* ***b****) papillae,* ***c****) lamellae.*

1.2.5: *Tubular density*

Tubular density is the number of tubules per millimetre^2 (mm^2) counted from a slide prepared from a transverse hoof wall section at 90° to the long axis of the tubules. The densities given by various authors has ranged from 7/mm^2 to 61/mm^2 (Reilly *et al,* 1998[a]). More recently Lancaster *et al* (2013) found tubule density in three regions of the wall; medial 11.2/mm^2, dorsal 10.4/mm^2, lateral 8.7/mm^2. These results were acquired from four 3 year old Quarter Horse cadavers and five live pleasure horses ranging in age from four to 28 years old, making it a small and heterogeneous sample. Presently there appears to be no data on foals or fetuses documenting tubular size or density.

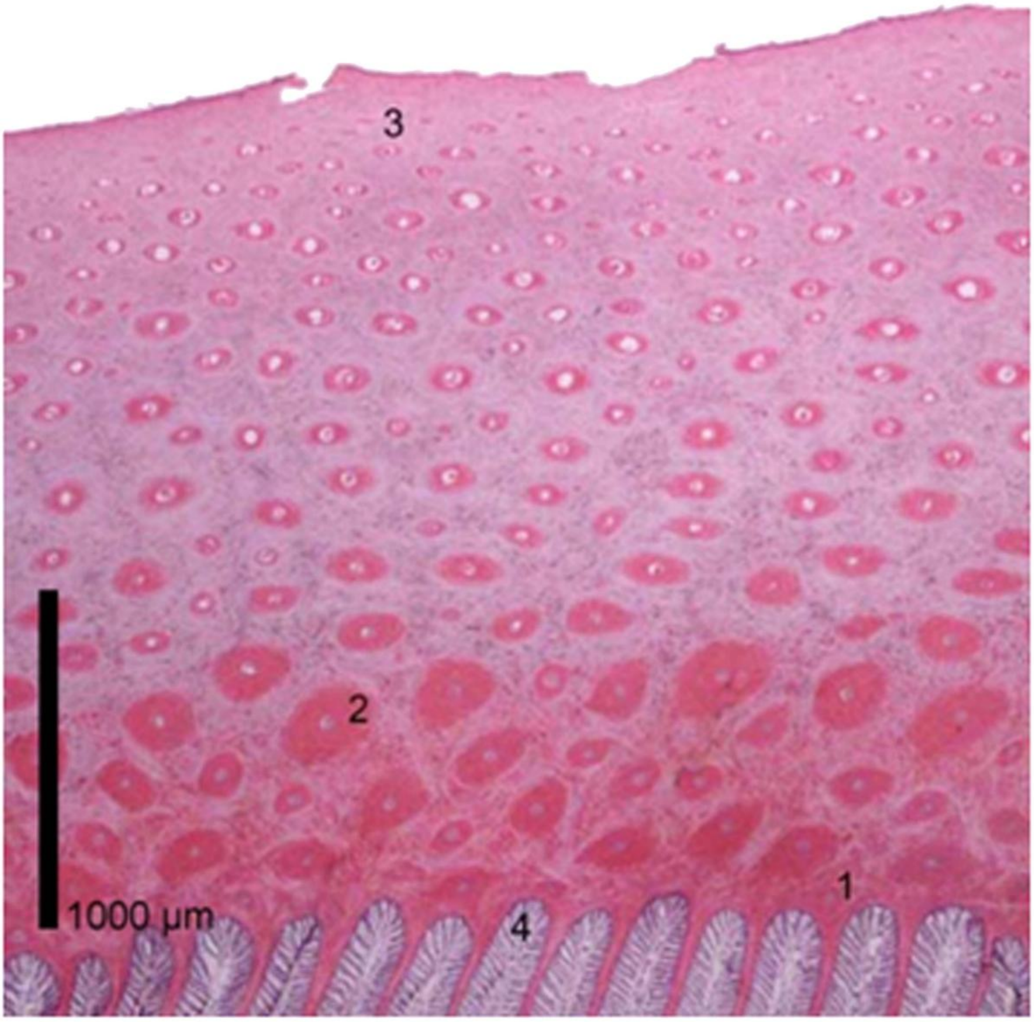


Figure 1.4: *a stained transverse section of the hoof wall of a 53 day old foal at x20 magnification; 1) small round axial horn tubules; 2) round larger horn tubules of the stratum medium; 3) small oval horn tubules of the abaxial hoof wall; 4) primary epidermal lamellae.*

1.2.6: *The hoof at birth*

During the second half of the 11 month gestation period the deciduous hoof capsule is constantly replaced from below by permanent hoof capsule so that foals have a fully developed hoof epidermis at birth (Bragulla, 1991). Foals stand immediately *post partum* and follow their mares. Feral horses have been recorded walking over 15 kilometres per day and domesticated horses in large paddocks over 7 kilometres per day (Hampson *et al*, 2010[a]).

Lancaster *et al* (2013) described the horn tubules as appearing to bud from the tips of the lamellae and although they were observing the hoof wall in mature horses, this may suggest a method by which hoof wall width increases. Mature Thoroughbreds have asymmetric hooves which have a thicker hoof wall medially, although it is not known whether they are born this way or alter during maturation (Roland *et al*, 2003). The tubular hoof wall structure of late fetuses and paediatric foals does not appear to have been studied and the manner by which the hoof wall thickens during maturation has not been explained. In Chapter 3 a microscopy study of the fetus and foal hoof wall that investigated the epidermis width and tubular structure of the *stratum medium* will be described.

1.2.7: *stratum internum*

The *stratum internum* is the connective region of the hoof wall which attaches the *stratum medium* to the distal phalanx. The distal phalanx and therefore the horse, is effectively suspended within the hoof capsule by the connective tissues of the *stratum internum*. The hoof wall and distal phalanx are attached by 550-600 interlocking primary dermal and epidermal lamellae (Pollitt, 1998). The primary epidermal lamellae (PEL) project from the distal phalanx towards the *stratum medium* at 90° to its tangent. In order to increase surface area to cope with the forces involved in suspension each PEL has 150-200 folds creating secondary epidermal lamellae which increase the surface area to an average 2.4 m^2 (Pollitt, 1998). This can be compared to the external surface area of the hoof wall in a mature Thoroughbred of approximately 250 cm^2.

1.3: Conformation

1.3.1: *Angles of the digit*

Front limb conformation and feet are usually considered from two views; frontal, described as dorsal and side view, described as lateral (Figures 1.5 and 1.6). In equine radiography a front view is termed as dorsopalmar (dorsoplantar in the hind) and a lateral view as lateromedial; these describe the direction of the radiographic beam (Meehan, 2002). The lateral view is used to assess both the hoof angle and its relationship with the pastern, termed the hoof pastern axis (HPA) (Figure 1.6).

Figure 1.5: *a dorsal view of the hoof capsule and pastern*.

As long ago as Ancient Greece, the horse's conformation and its quality of hoof have been considered of utmost importance in the selection of a sound horse. Xenophon, the great cavalry general and author (431-354 BC), explained the need for good hoof angles and pastern angle in *On Horsemanship* (Xenophon, 2008). More recently the practice of assessment by conformation when buying a racehorse has continued (Weller *et al,* 2006). The straight alignment of the HPA is considered to be ideal and varying from that alignment a flaw. A broken forward (positive) HPA, where the dorsal hoof is steeper than the pastern, is not considered to be as detrimental to soundness as a broken back (negative) HPA. A negative HPA creates more tension in the flexor tendons and shifts weight-bearing to the caudal hoof capsule (Balch *et al*, 1997).

1.3.2: *Maturation of angles*

Changing hoof and pastern angles have been noted in maturing Thoroughbred horses from the age of six months to three years (Anderson and McIlwraith, 2004). Weanlings were shown to have a steeper dorsal hoof wall angle (DHWA) which descended during maturation. The phenomenon of DHWA decent has not been recorded in TBs before six months of age or how this affects the HPA. The phalangeal axis is the alignment of the proximal, middle and distal phalanges of the digit. Their alignment cannot be viewed externally by eye and requires imaging using radiography. Although in healthy mature horses the phalangeal and hoof pastern axes align so that where, for example, the HPA is positive then it is reasonably assumed that the phalangeal axis is likewise also positive. In a previous study of weanling Warmbloods however, it has been shown that this is not so and the DHWA does not align with the parietal angle of the distal phalanx (Kroekenstoel *et al*, 2006). Chapter 4 will examine the early changes to DHWA, the HPA, and the phalangeal axis in foals.

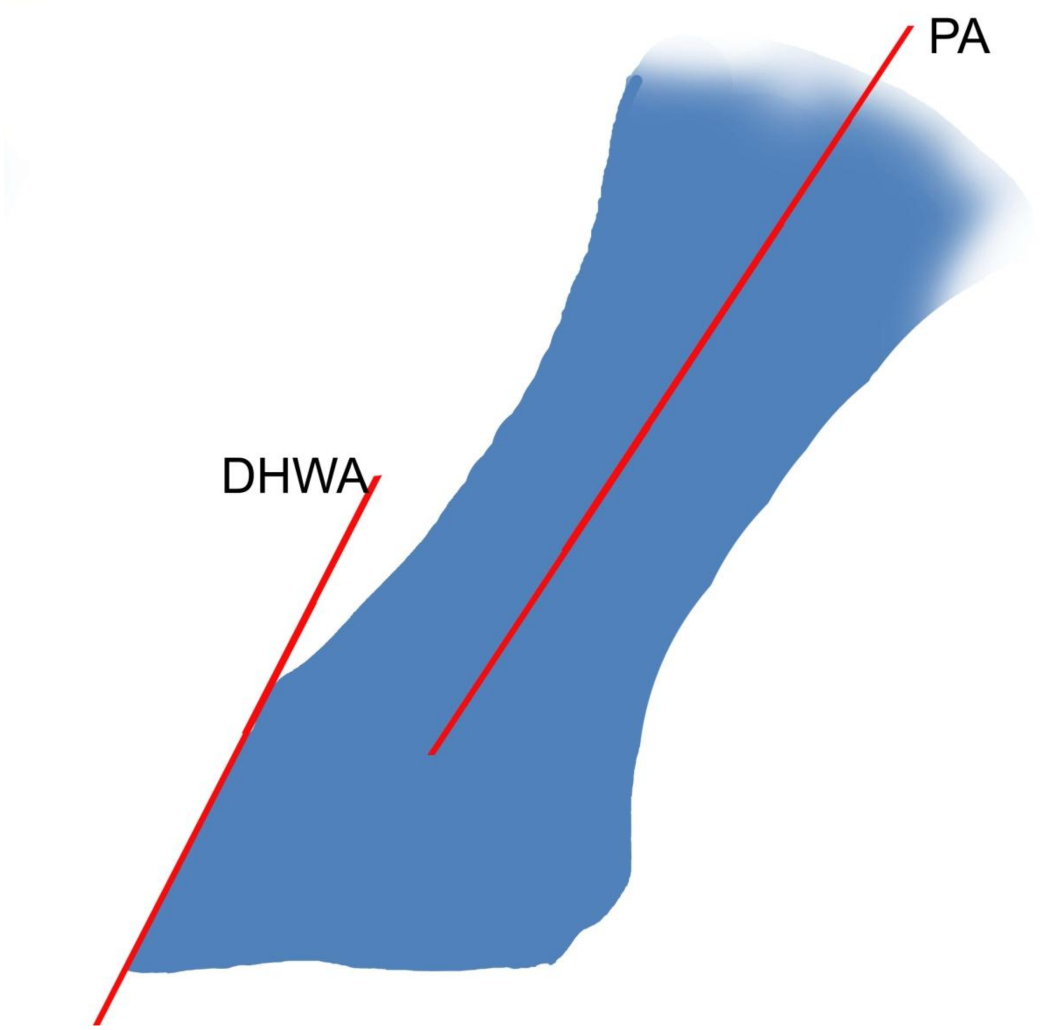


Figure 1.6: *A lateral view of the equine digit in silhouette;* ***DHWA****, dorsal hoof wall angle;* ***PA,*** *pastern angle; comparing the alignment of both gives the hoof pastern axis (HPA).*

1.4: Hoof Morphometrics

The racehorse Eclipse was measured by Charles Vial de Sainbel (1753-1793) *in vivo* and *in vitro*, to try to explain his absolute dominance on the racecourse. However, as Vial de Sainbel explained, *"Eclipse having been foundered many years previous to his death, his fore feet were much disfigured*", and therefore he was unable to take useful measurements of the hoof capsule. Farriers have long measured solar hoof dimensions to calculate the length of steel required to make a horseshoe, using various formulae depending on shoe size and type (Curtis, 1999; Beveridge, 2002). The simple method of morphometric measurement to acquire data seems to have been seldom used by researchers. This may be because there are few fixed points on the foot allowing accurate and reliable methods to be used in clinical and experimental work. The point at which measurement is taken requires a precise definition to allow the repetition of an experiment (Reilly *et al*, 1998[b]). Ruohoniemi *et al* (1997) in a study of cadaver specimens, measured the external foot, taking the length of the foot as being measured along the sagittal mid-line through the frog from toe to posterior central sulci. Even in a healthy frog this is not an exact reference point, in a thrush infected frog it is meaningless. One of the difficulties in measuring the hoof capsule and its external structures is that they are not geometric but irregular and parts merge into each other with ill-defined borders (Figure 1.7). Hooves are often broken at the distal margin or may be rounded-off by wear. If a researcher is measuring a hoof with a piece missing, a decision has to be taken as to whether to ignore the break and measure to where the hoof should be. Farriery work may also have changed the external hoof-shape, rasping away flares, dumping the toe or rounding off to prevent breakage. When the foot is shod this may cover structures that need to be seen and it certainly has an effect upon dorsopalmar angles (Moleman *et al*, 2005).

Figure 1.7: S*olar view of an untrimmed foal's hoof; 1) the frog; 2) the bars; 3) the sole; 4) bearing border of the hoof wall; although the constituent parts can be labelled, it is difficult to define their borders.*

1.4.1: *Measurement equipment*

There are a number techniques and tools that have been employed to measure the external form of the equine digit. Wilson *et al* (2009) used callipers and rule to collect morphometric measurements of the hoof and limb from 34 horses and related hoof width and height to limb height measurements. They concluded that asymmetry is present in horses and that the disparity in hoof width of the front pair may suggest unequal loading of the limbs. There appear to be no similar morphometric studies of the hoof in foals but in Chapter 4 solar hoof metrics are obtained from a cohort of foals.

1.4.2: *Defining measurement points*

Decurnex *et al* (2009) used a tape to measure the hoof circumference immediately below the coronary band*;* this study showed a decrease in coronary band circumference after a period of training and an increase after a period of paddock rest. Their methodology demonstrates the main problem of measuring hoof morphology; that the coronary band is difficult to define and rarely runs in a smooth direct line. Cripps and Eustace (1999) palpated immediately below the coronary band to find where it yielded and placed a wire marker there for radiological measurements. Reilly *et al* (1998[b]) considered, what they called "the reference hairline" (RH), a more reliable reference point than the junction of the hoof wall and coronary band. In a pilot study they found that the repeatability of measurements, using the RH was within 1%, whereas using the coronary band as a reference point through palpation was >4%. Reilly *et al* (1998[b]) burned an 'x' into the hoof wall as a permanent mark with which to measure growth over a period of months. Other researchers have scored a line across the hoof with the edge of a farrier's rasp to cause a mark that would remain visible until finally reaching the distal border (Faramarzi *et al*, 2009). Although the x method is probably more accurate and less damaging to the integument than scoring with a rasp, gaining client approval to use it on young Thoroughbreds would prove challenging. In Chapter 2 a pilot study is described where a novel technique for marking the hoof wall to allow hoof growth measurement is explained and this is later used in two studies in this thesis.

A hoof protractor, sometimes called a hoof gauge, is used to measure the angle and occasionally the length of the dorsal hoof wall (Martinelli and Ferrie, 1997). The use of hoof protractors has weaknesses, the most obvious being that the hoof wall is rarely straight at the dorsum and therefore requires the user to make a subjective judgement as to the placement of the bar along it. Trimming off any flaring where the hoof wall is concave before use, was recommended by Balch *et al* (1997). Where the hoof wall is convex, straightening it would be a risky procedure, involving thinning the width of the hoof wall. Using the gauge to measure hoof length has the previously stated problem of determining its exact origin at the coronary band. Moleman *et al* (2005) tested three commonly used types and a custom made device. They compared their results to digitally analysed radiographs and found that hoof protractors were unreliable. There appears to be no published work using any type of hoof protractor to measure the angles of foals, safety issues probably preclude their use and they were not used in studies described in this thesis.

1.4.3: *Radiographic measurement*

A radiograph is a two dimensional image produced by radio-waves passing through an object (Figure 1.8). The image seen as a negative, in tones of black and white, shows where the object most resists the passage of the radio-waves (white) and is said to be radio-opaque; where there is least resistance (black) it is described as radio-lucent (Meehan, 2002). It was not possible to gain internal measurements*, in vivo*, until radiography became available.

Figure 1.8: *A lateromedial radiograph of a foal with a fracture of the distal phalanx (red arrow); the fracture is more radio-lucent showing darker on the image.*

1.4.4: *Radiological studies*

Radiological studies are usually looking at lesions or lyses, which show disease, growth-related or conformational changes, or using radiographs to measure conformational features (Ruohoniemi *et al*, 1997; Dyson *et al*, 2011[a]; Beccati *et al*, 2011; Dixon *et al*, 2016). With analogue radiology, only the bones can be accurately defined but with digital radiographs finer detail is shown and some soft tissue structures, for example the periphery of the hoof wall, can be identified. It is difficult to accurately separate the joined dermoepidermis (integument) but the thickness of the combined structures can be measured from a LM radiograph (Sala, 2009). Collins (2011) called this measurement of the distance from the external DHW to the parietal surface of the distal phalanx the integument depth. When Cripps and Eustace (1999) measured this distance in healthy mature Thoroughbreds they recorded it as 16.1±1.75mm.

Radiographic studies of the equine foot have been used to measure the conformation, shape, angles and proportions of the bones of the digit (Caudron *et al*, 1998). The two most favoured views used are LM and standing dorsopalmar (DP) (Sala A, 2009). These two views have been used for studies of clinically lame horses and in conjunction with assessments of foot balance, photographic and morphometric measurements (Caudron *et al*, 1997; Dyson *et al*, 2011[a]; Collins *et al*, 2011).

1.4.5: *Radiography and foals*

There are few radiographic studies of foals and these have usually concentrated upon LM views showing DP conformation. Arnbjerg (1988) used LM views of nine foals, which had suffered a flexural deformity, described by the author as deep digital flexor tendon (DDFT) contraction. Some of the foals had an inferior check ligament desmotomy operation and others a Toe Extension shoe was used as a treatment. Arnbjerg (1988) reported that radiographs of the affected foals showed radio-lucent areas in the distal part of the distal phalanx and an increase in width and number of vascular channels. This group of nine cases were compared to a control group of four "normal" foals and an un-numbered, second group of foals that had been recorded as having hyperextension of the metacarpophalangeal joint. The conformational condition of hyperextension or hypoflexion tendons, is commonly called "toe-up" or "flaccid tendons" (Curtis and Stoneham 1999).

Kroekenstoel *et al* (2006) found in a study of 23 Warmblood foals that radiolographically the phalangeal axis and the HPA differed but the overall dorsopalmar alignment of the distal limb increased in a six month period. They also recorded the dorsodistal narrowing of the integument and discussed that this could not be explained only by a rotation of the distal phalanx relative to the hoof capsule and suggested that the phenomenon must involve other factors such as bone remodelling. A relative rotation of this type in mature horses, where the phalangeal axis is negative, is often called a reverse rotation (Bathe and Curtis, 2002) although Kroekenstoel *et al* (2006) did not use that term. Dorsodistal narrowing of the integument could be a phenomenon of foals younger than those in the Kroekenstoel *et al* (2006) study and answering the question of why it occurs may lead to a greater understanding of hoof development. A study of 22 foals using standing DP and LM radiographs and solar morphometrics in foals is reported in Chapter 4.

1.4.6: *Hoof renewal*

Hoof wall renewal in all ages of horses has been poorly reported. Only Josseck (1991) has measured and calculated the time for the hoof of mature horses to grow from its proximal origin to the distal border at the toe. In her study of Lippizzaner horses this was found to be 11 months. Hoof growth rates in mature horses have been reported without stating hoof wall renewal time (Reilly *et al*, 1998[b]; Faramarzi *et al*, 2009). A number of authors have speculated that the hoof wall grows faster in young horses but only two have measured hoof growth rates in foals (Smallwood *et al*, 1989; Butler and Hintz, 1977). The study that Smallwood *et al* (1989) carried out on American Quarter horse foals consisted of only four and these were paediatric age. Although Butler and Hintz (1977) described the Shetland ponies, that they measured as foals, in this thesis their age would have defined them as weanling/yearlings. Neither of these studies investigated the time required from birth to replace the hoof capsule or wall.

Horses often suffer partial hoof wall avulsion which may cause lameness and threaten sales value (Parks, 2008). When there are lesions to the hoof wall it is useful to have an estimation of renewal time. The first study described in Chapter 5 measured the time taken for the fetal hoof of newborn foals to grow to the bearing border and be replaced by hoof grown since birth. The second study in the same chapter reports the measurement of hoof renewal in an older group of weanling/yearlings.

1.4.7: *Plastic deformation*

Changes in equine hoof capsule shape have been linked to unsoundness (Wilson *et al*, 1998; Dyson *et al*, 2011[b]), but the causes of such changes are less well understood. Although lameness and hoof distortion have been related there is usually a debate regarding which is cause and which effect (Holroyd *et al,* 2013). Over time, the shape of the hoof capsule can alter, with some changes leading to non-desirable foot shapes. These include club foot, collapsed heels, flared walls, sheared or contracted heels and concave toes

(Bowker, 2003; Hunt, 2006). Currently, the factors influencing hoof capsule shape change are thought to be associated with; hoof growth, wear at the bearing border, farriery, and plastic deformation but little scientific evidence exists to substantiate these theories.

1.4.8: *Weight-bearing and load*

From birth the hoof wall grows distally from the coronary epidermis whilst under load. Horn tubules are produced from the papillae which merge with the intertubular horn from the germinal epithelium (Bowker, 2003). Loads consist primarily of a tensile force applied by the deep digital flexor tendon to the distal phalanx and mass multiplied by acceleration, due to the descending bodyweight, with resulting ground reaction forces acting upon the solar surface of the hoof which is mainly the bearing border of the hoof wall (Hobbs *et al*, 2004). The weight of the horse is suspended within the hoof capsule via the lamellae and the majority of weight-bearing load is transferred through the hoof wall to the ground. The frog has a limited weight-bearing function as does the sole, which because the solar venous plexus lies between the dermal sole and the distal border of the distal phalanx, direct contact with the ground may compromise blood circulation (Balch and Butler, 1997).

1.4.9: *Hoof wall structure*

In order to carry load the hoof wall is designed with a tubular laminated structure which, in healthy mature hooves, remains parallel to the dorsal parietal surface of the distal phalanx while descending to the bearing border. The hoof, like most biological materials is neither completely viscous nor completely elastic and is therefore described as viscoelastic. The hoof wall is both viscoelastic and anisotropic in nature, varying across the *stratum medium* from axial to abaxial hoof wall and also from dorsum toward heel (Kaspasi and Gosline, 1996; Davies, 2002; Hobbs *et al*, 2004). Water content is related to stiffness so that the inner hoof wall, closest to the sensitive structures, is more flexible. The thickness of the hoof wall reduces dorsum to heel and this means that functionally the quarters are more flexible than the dorsum (Douglas *et al*, 1996).

1.4.10: *Differential hoof growth*

Changes in hoof shape may be caused by differing hoof growth rates at various sites around the hoof capsule (Faramarzi *et al*, 2009). An example of differing hoof growth might be a club foot, where these changes may be the result of the hoof wall at the heels growing faster than at the toe; the toe growing more slowly than the heel or a combination of these. It has been speculated that increased blood flow may influence hoof growth but this was not found in two studies of 18 Standardbreds and eight mixed breed horses (Glade and Salzman, 1985; Faramarzi *et al*, 2009). In normal development the hoof capsule changes shape from an inverted cone as a foal to resemble an obliquely truncated cone in the mature horse (Kaspasi and Gosline, 1996; Bidwell and Bowker, 2006).

1.4.11: *Hoof wear*

Hoof wear is a product of the environment, the amount of exercise undertaken, and durability of horn. Wear from the substrate mainly affects the bearing border with some wear of the abaxial dorsum. Assumptions of hoof shape have been based on the observed wear of American Mustangs' hooves where the dorsodistal hoof wall is radically rounded and wall at the quarters arched so as not to be in contact with level ground (Ovnicek, 2003). However, more widely based studies of feral horses in Australia and New Zealand found that hoof shape in feral horses varied according to their environment, from similar to the American Mustang to flaring at the quarters and dorsum (Hampson *et al*, 2010[b]; Hampson *et al*, 2013).

1.4.12: *Farriery*

Although farriery has been implicated in changing hoof shape and can bring about substantial transformation in a short time, it seems unlikely that farriery alone can be responsible for the wide variation in hoof shapes. Farriers can remove different depths of hoof wall at varying points around the bearing border and remove outer hoof wall by rasping, causing a change in shape (Kummer *et al*, 2006; Dyson *et al*, 2011[b]). However, unless farriery is frequent then these changes may be temporary as the greatest influence upon hoof capsule shape is limb conformation and the consequent loading (Glade and Salzman, 1985).

1.4.13: *Deformation of horn*

It has been suggested that plastic deformation is a factor in shaping hoof capsules, which is possible considering that the hoof capsule is a viscoelastic composite of tubular and intertubular horn which changes in thickness and density with location (Kaspasi and Gosline, 1996; Dyson *et al*, 2011[b]). This is partly due to the density of horn tubules: in mature horses the axial horn tubules of the *stratum medium*, have a density less than 11/mm^2 compared to the abaxial *stratum medium*, which have a density of greater than 22/mm^2. The differing densities of horn tubules around the hoof wall are also not uniform (Lancaster *et al,* 2013). The tubular density of the hoof wall at various sites has not been measured in foals. In Chapter 3 a study is reported that measured the density of horn tubules around the hoof wall in fetuses and foals at similar sites to Lancaster (2013).

1.4.14: *Elasticity of horn*

The mechanics of elastic hoof have not been fully explored but Nickel (1938) suggested that horn tubules have a layered spiral structure that can compress telescopically (Figure 1.9), and more recently Bellenzani *et al* (2012) found that the hoof wall compressed during locomotion as the stance phase progressed and more weight was imposed on the hoof wall. External strain gauges applied to the hoof wall *in vivo* have shown that strain magnitudes increased with toe length and toe angle, although these findings contradicted *in vitro* findings (Thomason, 1998). Thomason (1998) also found that strains were inversely proportional to medial and lateral angles and that when strains were recorded during the horse moving on a turn, the hoof quarter on the inside of the strain experienced 40% more strain than during straight line exercise. Principal strain became more compressive with increased dorsal hoof wall angle.

Figure 1.9: *Nickel (1938) suggested that horn tubules had a concentric spiral structure which allowed elastic compression during loading.*

1.4.15: *Role of horn tubules*

Kaspasi and Gosline (1997) suggested that the role of horn tubules was to provide a method of crack prevention for the *stratum medium* and also that horn tubules appeared to affect neither the hydration rate nor hoof wall flexion and had only a minor effect on thermal conductivity. It is surmised that plastic deformation may be a combination of bending of the horn tubules and material compression of tubular and intertubular horn. Whereas bent tubules can be observed, compression of intertubular and tubular horn cannot and this may be why it has not been previously considered.

1.5: Hoof growth and hoof renewal

1.5.1: *Hoof wall growth*

The generation of hoof horn has been described earlier in this chapter. The hoof wall moves distally at a speed which varies according to age and may also be influenced by breed, environment and hoof shape (Glade and Salzman, 1985; Smallwood *et al*, 1989*;* Josseck *et al*, 1995). Because of these factors and the additional variation of hoof size, the time taken for complete hoof wall renewal also differs. Currently there is no explanation as to why age should affect hoof growth and hoof renewal. Chapter 5 of this thesis describes two studies of hoof renewal times for Thoroughbred foals, weanlings, and yearlings and explores the question of why hoof growth rate appears to be faster in young horses compared to mature horses.

1.5.2: *Hoof growth rate*

Hoof growth rate (HGR) has been measured in mature horses but only in a few foals. The HGR of four Quarter Horse foals, aged from 77 to 119 days of age, was 0.42mm/day (Smallwood *et al*, 1989) compared to 0.25mm/day on a limited diet and 0.38mm/day when fed *ad libitum* in 14 Shetland Ponies with a mean age of 262 days (Butler *et al*, 1977), and 0.21mm/day for six mature (>4 years) Thoroughbred/Irish Draught crosses (Reilly *et al*, 1998[c]). While these studies were experimentally different they do imply a slowing HGR with ageing. Other domestic ungulate species have had growth rates reported showing a slowing with aging. Porcine hoof has been recorded at 0.43mm/day at one to 12 weeks of age, reducing to 0.18-0.21mm/day at 1-1.5 years old; bovine hooves, under one year of age, have been at recorded 0.23mm/day compared to 0.16mm/day in mature cattle (Geyer, 1979; Landerer, 1999; Stern, 2000).

1.5.3: *Hoof angle*

Changes in toe length by trimming have also been shown to influence hoof growth rate, as steeper angled toes had a slower HGR, 0.22mm/day, than those with the lower toe angles, 0.26mm/day (Glade and Saltzman, 1985). Since hoof shape has been shown to alter compression forces in the hoof wall (Thomason *et al*, 1998) this may mean that HGR is influenced by hoof angle and loading.

1.5.4: *Hoof avulsion*

Horses often suffer partial hoof wall avulsion which may cause lameness and threaten sales value (Parks, 2008). When there are lesions to the hoof wall it is useful to have an estimation of renewal time. In mature Lipizzaner horses hoof renewal has been calculated as 335 days (Josseck *et al*, 1991). There appear to be no other studies which have given hoof renewal times for other breeds or ages. The knowledge of hoof renewal times would seem to be of similar importance as HGR and more useful in clinical situations. Little scientific evidence exists on factors that influence hoof capsule changes in shape; while hoof growth rate and renewal times have not been recorded in foal, weanling and yearling Thoroughbreds.

1.5.5: *Hoof renewal from birth*

Thoroughbred foals stand within minutes of birth on hooves developed *in utero* which are covered by eponychium, also known as deciduous hoof. This is shed within a few days in a healthy foal (Bragulla, 1991). From birth a growth ring is present in the hoof wall parallel to the coronary band. Smallwood *et al* (1989) noted that in LM radiographs of young foals an indent in the hoof wall, which he described as a ‘groove’, was seen on all four hooves, marking the event of foaling (Figure 1.10). The horn proximal and distal to this line often differs in colour (Ellis, 1998). Butler and Hintz (1977) studied the rate of hoof growth of 14 Shetland pony foals of eight to eleven months of age and did not mention this line, which suggests that either they did not think it remarkable or failed to notice it because it had disappeared by the age of measurement.

Chapter 5 of this thesis will describe two studies of hoof renewal times for Thoroughbred foals and weanling/yearlings and explore the question of why hoof growth rate appears to be faster in young horses compared to mature horses. In Chapter 6 measurement of hoof growth, from this age, is combined with simultaneous data testing of hoof wall compression to provide information that will further our knowledge as to how changes in hoof shape occur during development.

Figure 1.10: *A foal of approximately two months age showing the foal hoof crease (FHC) (red arrow), marking the time of birth.*

1.6: Weight-bearing and loading

Although healthy foals stand within minutes of birth they are unstable and adopt a wider stance mediolaterally to overcome their weak myotendinous development and poor postural control (Adams and Mayhew, 1984; Nauwelaerts *et al*, 2013). Foals appear to be born with symmetrical hooves (Bidwell and Bowker, 2006) and they alter in size and shape during normal healthy development. It is not known what part loading plays in typical hoof development. From the author's experience, neonatal foals appear to load their heels more than the toe and by one month most appear to load evenly, however this has not been measured (Figure 1.11). Where a foal has an obvious acquired flexural deformity (AFD), for example affecting the distal interphalangeal joint, weight can be seen to borne at the dorsodistal bearing border with the caudodistal bearing border not in contact with the ground (Owen, 1975; Curtis *et al*, 2012). Where the hoof distorts, to become a club foot, it is not known whether the hoof wall at the heel is growing faster than at the toe, the toe more slowly, a combination of these or that there is another phenomenon affecting horn shape changes. Loading and weigh distribution is further discussed later in this chapter.

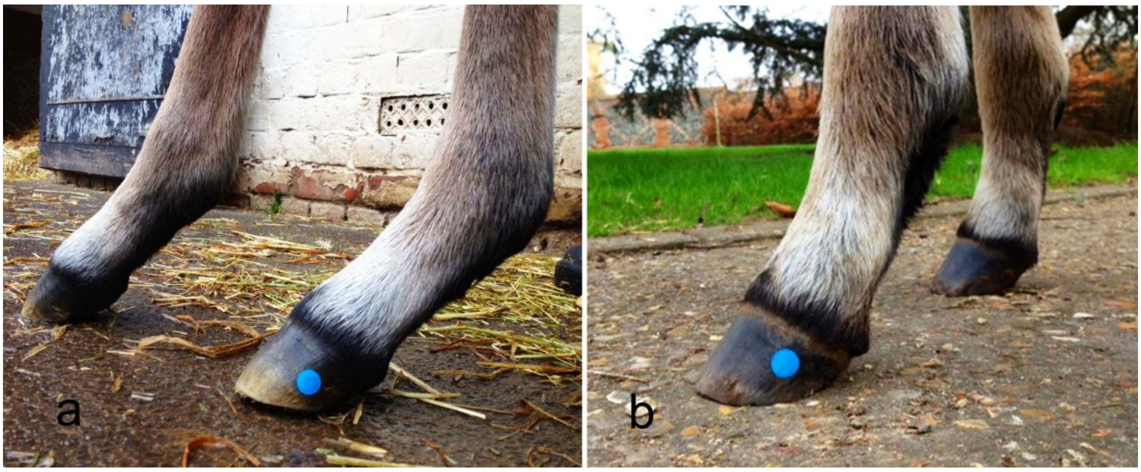


Figure 1.11: *A 12 hour old foal (a) typically has a low HPA and appears to lean back on the heels; 14 days later the same foal (b) appears to have a more upright HPA and to bear weight more evenly dorsopalmarly.*

1.6.1: *Photometry:*

Photographic images have an advantage over radiographic images in that they do not require expensive equipment nor is there a hazard from radiation (White *et al*, 2008). White *et al* (2008) compared measurements taken from photographic images of 20 Thoroughbreds in training to radiographic images of the same horses, using image measuring software, and evaluated precision between images and users. They concluded that excellent precision was found between operators; ≥0.90, SD ≤0.10, indicating that radiography and photography may be used interchangeably and that acquisition and analysis of photographic images is an appropriate method for the objective measurement of foot conformation. Hoof growth in this thesis was recorded using digital photography, and measured using the same software.

1.6.2: *Visual assessment*

Descriptions of visual assessment of the horse's leg and foot conformation are some of the earliest existing recordings of literature on the horse. Xenaphon (430-354 BC) is often quoted for his advice on the buying of horses, although he may be parroting the words of the earlier Simon of Athens (c. 480 BC) when he advised on concave soles, upright hooves but pasterns not as high as to jar the rider. Vial de Sainbel (1797) stated that the pastern should be in alignment. Few observational studies of conformation have been undertaken, possibly for the simple reason that modern science requires objective quantitative measurement. Mawdsley (1996), in a study of two and three year old Thoroughbreds, identified 27 conformational traits, although only six were objective measurements and 21 subjectively scored assessments. In his study dorsal hoof wall angle, which was measured, was significantly associated to age.

1.7: Equine hoof development

The horse has a gestation period of 340 days, giving birth to single foals which stand and suckle from their mares within minutes. In nature, mares and foals move with their herd from their first day *post partum*. At birth the foot of the foal must be capable of fulfilling the same functions as a mature horse's foot; protecting the inner sensitive structures, bearing weight, reducing concussion, resisting wear, and providing grip and purchase for locomotion.

1.7.1: *Development of the hoof in utero*

During the embryonic period limb formation begins in the limb field, as mesenchymal cells from the lateral plate mesoderm proliferate causing the ectoderm to bulge forming the limb bud (Bragulla, 2003) (Figure 1.12). By 65 days the hooves can be readily identified even though the limbs contain no bone (Figure 1.13). By 85 days bone can be identified radiographically within the lower limb (Ellis, 2002) (Figure 1.14). In later pregnancy the hooves develop the hoof covering eponychium to protect the uterine wall (Figure: 1.15). At birth the hoof capsule is covered by eponychium, also known as deciduous hoof. It is a soft gelatinous type of horn generated from the coria of periople, sole and white line. In healthy foals the deciduous hoof is shed during the first few days of life once the foal stands and walks, when its purpose, the protection of the uterus, has ended (Bragulla, 1991). Changes to the microscopic structure of the hoof wall of the fetus, neonate and paediatric foal are examined in Chapter 3.

Figure 1.12: *A 34 day old equine embryo with the LF forelimb bud evident.*

Figure 1.13: *A 65 day old equine fetus with hooves, although at this stage there is no bone formation within the limbs.*

Figure 1.14: *A radiograph of an 85 day old equine fetus with some bone formation within the limbs*.

1.7.2: *Material properties of hoof horn*

Horn is not living tissue as it has neither a vascular structure nor a nervous system (Tombolata *et al,* 2010). A consequence of the hoof wall's viscoelasticity is that fracture toughness decreases with increased strain (Kaspasi and Gosline, 1996). The hoof wall is a complex reinforced composite consisting of tubular, intratubular and intertubular horn. Approximately half the hoof wall consists of tubular horn filled with intratubular horn and this is surrounded by intertubular horn. These three structures combined to form the *stratum medium* which is anisotropic in nature. Intertubular horn is fashioned from keratinocytes as previously described which are at right angles to the horn tubules, giving the *stratum medium* its anisotropic quality. Hoof keratin, one of the toughest biomaterials known, is organised in a structure which makes it well suited for its role in protection and wear resistance (Bertram and Gosline, 1986).

1.7.3: *Fracture toughness*

Bertram and Gosline (1986) showed that, despite the commonly held belief, there was no difference in fracture toughness in pigmented and un-pigmented hoof wall and that the distal hoof wall had less resistance to fracture than the central to proximal wall. This may suggest material fatigue as the distal hoof is the oldest. The heterogeneous tubular structure changes from axial to abaxial in the *stratum medium* and this may be a method of transferring load and dissipating forces from locomotion through the hoof wall to the suspensory apparatus of the hoof and has been shown to prevent cracks migrating axially (Figure 1.4). Fracture toughness in the hoof wall has been measured using the *J*-integral method which is used for materials which show plastic deformation. Kaspasi and Gosline (1997) found *J*-integral values ranging from 5.5 to 7.8 kJ m^{-2} through the wall thickness.

Figure 1.15: *The foot of a neonate covered with deciduous hoof (eponychium).*

1.7.4: *Elastic deformation of hoof*

Hoof wall deformation occurs during the stance (weight-bearing) phase of locomotion with both the dorsal and lateral aspects moving towards the axis of the digit with deformation increasing proximally (Burn and Brockingham, 2001). At the heels the hoof wall responds to hoof capsule loading by expanding during the first 70-80% of the stance phase and then recoiling to contract at break-over (Yoshihara *et al*, 2010).

1.7.5: *Tendons of the equine leg*

The front leg of the horse has four major tendons below the carpus, these are; the common digital extensor, lateral digital extensor, deep digital flexor, and superficial digital flexor (Figure 1.16). These tendons show elasticity and great tensile strength and transmit strain from their muscles, extending and flexing the metacarpophalangeal and interphalangeal joints (Murray, 2002). The two flexor tendons are supported by accessory ligaments; the superior carpal check ligament or accessory ligament of the superficial flexor tendon and the inferior check ligament or accessory ligament of the deep digital flexor tendon. The myotendinous units of the deep digital flexor and the superficial digital flexor play an important role during the stance phase of locomotion. In mature Thoroughbreds *in vitro* testing of the flexor tendons and their accessory ligaments showed that the accessory ligaments transmitted more force to the distal tendon than the muscle. Linear stiffness was 1.93±0.11 kN/cm for the superficial digital flexor accessory ligament-tendon unit and 2.47± 0.11 kN/cm for the deep digital flexor tendon accessory ligament-tendon unit (Swanstrom *et al*, 2004).

1.7.6: *Hoof capsule shape change*

The symmetrical paired feet that foals are born with would appear to alter in shape during development. By one year of age front hoof capsules are a variety of shapes which are often mismatched with the opposing foot and show asymmetry within individual feet (Bidwell and Bowker, 2006). By maturity (≥3 years) typically the hoof is wider laterally than medially and the hoof angle to the ground is more oblique on the lateral side (Roland *et al*, 2003; Faramarzi *et al*, 2009). Hoof capsule deformation has been attributed to hoof wear, farriery, differing rates of hoof growth around the coronary band, and plastic deformation (Kummer *et al*, 2006; Dyson *et al*, 2011[b]). Although hoof wear and farriery undoubtedly have an influence upon hoof shape, unless persistently applied, changes may be temporary. Differential hoof growth rates at sites around the coronary band and plastic deformation of hoof horn have not been previously measured. Front hooves also often differ in dorsal hoof wall angle and this may be due to a number of factors including; a flexural deformity, stance, or injury (Owen, 1975; van Heel *et al*, 2006; Dyson *et al*, 2011[b]). The processes by which hoof capsule shape change occurs are poorly understood.

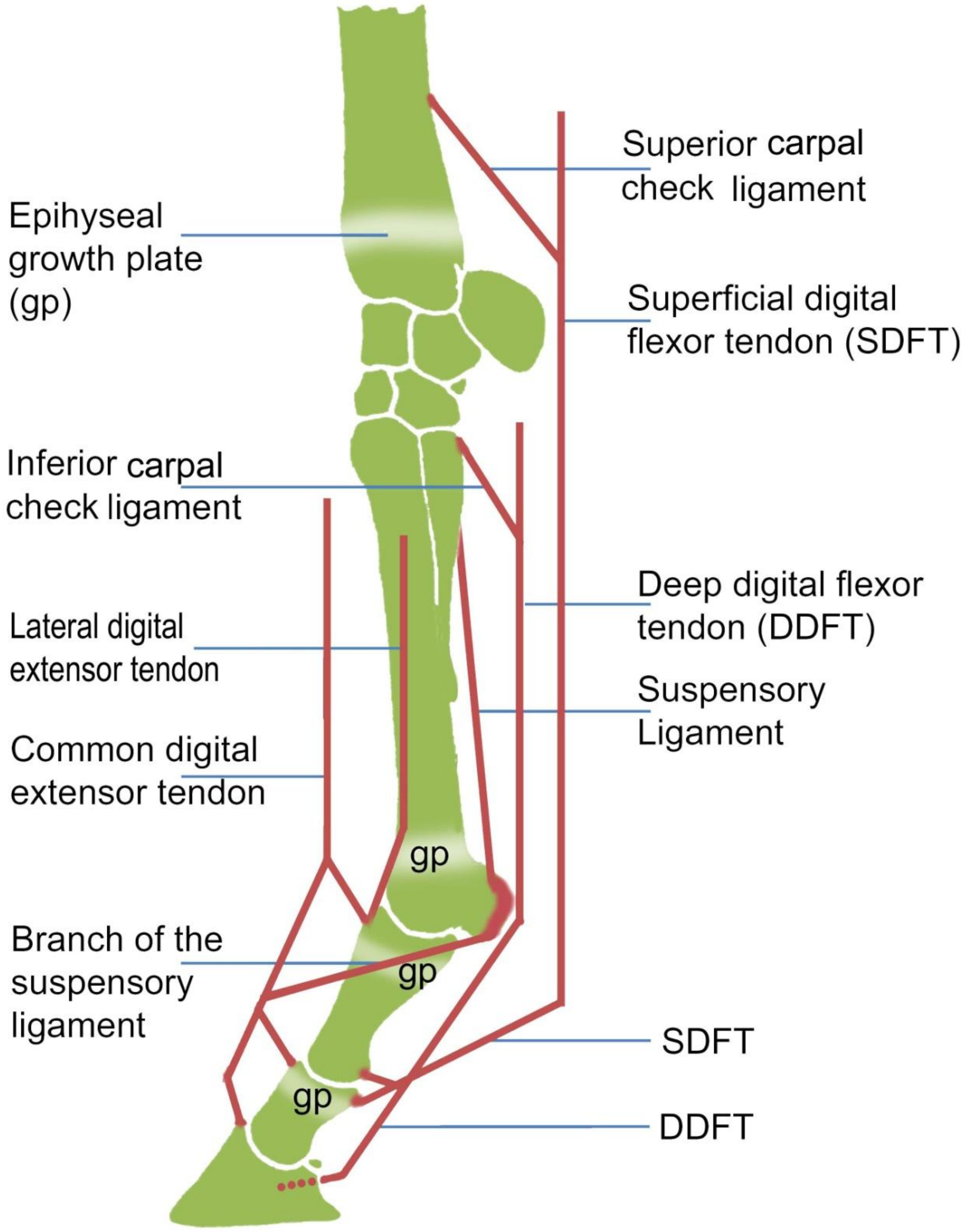


Figure 1.16: *Schematic diagram of a front limb of a foal at one month; the four tendons of the front leg found below the carpus with accessory check ligaments, the suspensory ligament and sites of epiphyseal growth plates (GP).*

Shape change in the hooves of mature horses has been described as plastic capsular deformation (Faramarzi *et al*, 2009; Dyson *et al*, 2011[b]). This would imply that the hoof wall compresses during the decent from its generation at the coronary corium to the bearing border and that this affects hoof growth rate. It has been suggested that hoof growth rate (HGR) may vary at sites around the hoof capsule and that this may be a factor in differing hoof shape. Although Frackowiak and Komosa (2006) measured hooves at five points around the hoof wall, they did not report findings relating to differential growth. Faramarzi *et al* (2009) found, in mature Standardbreds, that during a 17 week period HGR was; at the dorsum 0.22mm/day, lateral 0.31mm/day, and medial 0.32mm/day. If hoof compression occurs during decent then the amount of horn generation at the *stratum germinavatum* of the coronary corium and the rate that it descends to the bearing border may not be synonymous. Chapter 5 describes a study of 12 weanling/yearlings, designed to test whether the hoof wall compresses after generation and Chapter 6 will record a study of hoof growth and hoof loading in 27 foals.

1.7.7: *Acquired flexural deformity of the distal interphalangeal joint*

Acquired flexural deformity of the distal interphalangeal joint (AFDdipj) is a recognised condition of the fore limbs causing the heels to not contact the ground and the foal unable to extend the joint to a normal angle (Figure 1.17). In untreated cases this ultimately leads to club foot. The onset of the initial ‘heels up’ conformation appears to be rapid at 24 to 48 hours (Owen, 1975). AFDdipj has been recorded in many horse breeds and also donkeys. Although the causes are not fully understood the signs are well recognised (Arnberg, 1988; Daniels *et al*, 1990; Kidd and Barr, 2002). Many authors have stated the timing of this condition without data, from as early as one month to 8 months (Fackelman, 1979; Adams and Santschi, 2000; Kidd and Barr, 2002), but one study recorded its incidence in Thoroughbred foals of one to four months (Curtis *et al*, 2012). There are many recognised treatments for this condition including; 1) early use of oxytetracyline, 2) restriction of exercise, 3) application of a Toe Extension shoe, 4) inferior check ligament desmotomy, 5) splinting. Treatment often involves a combination of two or more of these (Curtis, 1992; Munroe and Chan, 1996; Warmsley *et* al, 2011; Compston and Payne, 2011).

Figure 1.17: *A foal affected by AFDdipj is unable to extend its distal interphalangeal joint in both front legs and bears weight on the solar dorsum.*

1.7.8: *Weight distribution of the limbs*

The weigh distribution on the front limbs at standing has been shown as 58.7% ± 3% of body weight (Hobbs et al, 2014). This reinforces the widespread view that in horses the distribution between front and hind limbs is proportionately 60:40. This may be one factor in explaining the differing foot shape and size between front and hind hoof capsules in an individual. The front foot tends to be rounder and wider than the hind which is more oval in shape and pointed at the toe. The hoof bears weight mainly on the hoof wall with some loading of the sole at the peripheral margin where it connects via the white zone to the hoof wall. Weight-bearing and load distribution have been studied dynamically and quasi-statically (Hood *et al*, 2001; van Heel *et al*, 2004; Gomes-Costa *et al*, 2015). The distribution of load is not even over the solar hoof and this varies according to hoof trimming style and timing (Hood *et al*, 2001). If hoof loading is uneven and it influences plastic compression then this in turn may affect hoof growth in such a manner as to change capsular hoof shape. The weight-bearing and quasistatic load distribution of paediatric foals' feet is not known.

1.7.9: *Pressure mats*

Pressure mats are used to ascertain loading patterns, showing the distribution of weight-bearing across the solar surface of the foot. A commonly used system has a measuring surface of 98 x 32.5cm containing 8192 conductive sensors. Data is collected at 50Hz producing both visual and numerical interpretation. Force plates are often used in conjunction with pressure mats in human medicine and sports analysis to measure force vectors during gait and have been used in equine science. Both systems give quantitative information about the centre of pressure while moving or standing (van Heel *et al*, 2004). Illustrations and a description of pressure mat use are found in Chapter 2. Chapter 6 will describe a study which investigated solar hoof loading, hoof growth, and hoof compression, in healthy foals and foals with AFDdipj.

1.8: The Thoroughbred breeding industry

The Thoroughbred was originally developed in 17th century England, when native mares were crossbred with three imported stallions; the Byerley Turk, the Darley Arabian, and the Godolphin Arabian. All modern Thoroughbreds can trace their pedigrees to these stallions in the closed stud book which dates back to 1791. Worldwide there are over 100,000 Thoroughbred foals registered each year, with approximately 30,000 in the United States of America and approximately 8,000 in the United Kingdom. Although breeding farms are scattered in major breeding countries there are concentrations, for example; Kentucky in America, the Hunter Valley in Australia, and Newmarket in the UK. Newmarket has approximately 90 stud farms of varying size from birthing (foaling) no foals to some foaling more than 100 foals each year. Many broodmares stay in Newmarket all year round and are described as boarding mares, others travel in from other parts of the UK and overseas to give birth to their foals and then be covered (impregnated) by a stallion. The foaling season in the northern hemisphere begins on the first day of January and ends in mid-June; however few foals are born before the third week of January and few after May. Foals are weaned from their dams at approximately five months of age. Some are sold as foals at the end of the year and many as yearlings at the approximate age of 20 months. Some are not sold as the owners breed them to keep as racehorses for themselves.

1.9: The aims of this thesis

The project described in this thesis comprised of five separate studies which aimed to answer some fundamental questions of hoof wall growth and hoof shape during development in Thoroughbreds from late term fetus, through paediatric foal, weanling and yearling phases. The aims of the microscopy study in Chapter 3 were to find the means by which the epidermal hoof wall increases in thickness and compare the known tubular structure of mature horses with fetuses and paediatric foals. The aims of the radiographic and morphometric study reported in Chapter 4 were to document the morphometrics of the hoof capsule and phalanges in Thoroughbred foals and to quantify changes in conformation and morphometrics that occur due to growth and development in the first year of life. The studies reported in Chapter 5 aimed to document hoof renewal in newborn foals and weanling/yearlings, to measure hoof growth and hoof length in weanling/yearlings and to determine whether the hoof wall compresses plastically over time. The aims of the study reported in Chapter 6 were to quantify and compare hoof growth and hoof compression in the hoof wall, solar hoof loading, and dorsal hoof wall angle, in healthy foals and foals with an acquired flexural deformity affecting the distal interphalangeal joint.

1.10: Hypothesis

Bidwell and Bowker (2006) suggested that foals are born with symmetrical paired hooves and Dyson *et al* (2011[b]) stated that changes in loading will result in changes in hoof shape. Therefore, it was hypothesised that changes in hoof shape in Thoroughbred foals will be influenced by changes in loading patterns.

Chapter 2: Development of methods

This chapter describes the development methodology required to achieve objectives that fulfilled the aims listed in Chapter 1. Some methods were further improved for the full studies reported in Chapters 3 to 6 so there is some repetition.

2.1: Pilot studies

All of the five studies reported in this thesis were piloted prior to commencement of the full studies. Equipment and methods were tested to ascertain accuracy and safety. Although the author is an experienced farrier, familiar with lifting and holding foals' hooves, the requirements for handling young horses, while creating marks, measurement and photography, needed identifying and refining. The type of statistical testing was explored and numbers required for statistical power was estimated. The number of horses used in the studies was tempered by the knowledge that these were highly valuable animals and the agreement and cooperation of the stud farms was needed.

2.2: Histological methodology for microscopy study

2.2.1: *Skill training*

Initially the author spent a week at the Institute for Veterinary Anatomy, The Free University, Berlin, learning how to sample hoof from a cadaver specimen, cut horn using a microtome, and prepare slides. The leg of a mature horse was taken from a cabinet freezer and disarticulated at the fetlock. An industrial size band-saw was demonstrated showing the initial transverse cut and a second cut parallel to the direction of growth. The transverse slice was then sectioned at the point of interest leaving lamellae intact but removing bone. The sample was then stored in 10% neutral buffered formalin in a universal container. Due to the time required to prepare embedding in paraffin before cutting a prepared sample was used to practice. The author was trained in manual cutting using a microtome, careful recovery of cut samples to distilled water and selection and lifting to the slides. The process of staining and preservation of slides was explained.

2.2.2: *Methodology practice*

At the Beaufort Cottage Laboratory, Newmarket, the techniques learned in Berlin were discussed with the head of pathology and the technician with histology experience. These techniques were then practiced. Two front digits from a cadaver foal aged six months were cut using a large fixed post-mortem band saw. Two orientations of cut were tried; the first, parallel to the bearing border and the second, parallel to the coronary band (Figure 2.1).

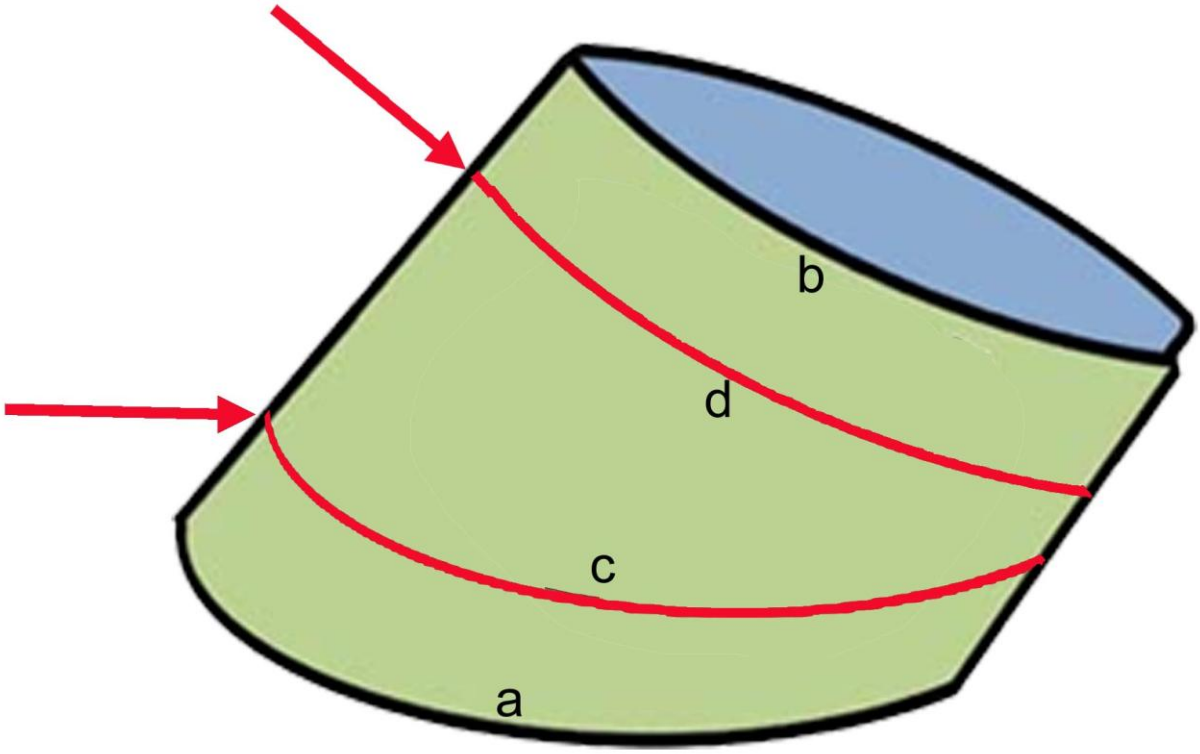


Figure 2.1: a*) the bearing border of the hoof capsule; b) coronary band; c) a transverse cut parallel to the bearing border; d) a transverse cut parallel to the coronary band; arrows show direction of cut.*

It was apparent that the cut parallel to the bearing border distorted the samples due its obliquity and the cut parallel to the coronary band (CB), in effect cutting at 90° to the long axis of the horn tubules at the dorsum, did not. From the cut parallel to the CB the author trimmed the samples to size, marked them for vertical orientation and preserved them in 10% neutral buffered formalin, within labelled 27 centilitre universal containers. At this point embedding the sample blocks in paraffin was carried out by an experienced technician (Appendix A). The samples were then cut at 4µm using a bench microtome, prepared as slides and stained with haematoxylin and eosin. Initially the hoof proved difficult to cut and different softening methods were tried. Treating the embedded hoof sample with a hair removal product[1], for two days before cutting, improved the quality of slide samples. The slides were examined under various magnifications, using a Nikon Eclipse 50i microscope with camera[7], images were recorded at x20 and x40, magnification for analysis and measurement and saved as lossless Jpeg files with an image embedded calibration. The calibration was checked for accuracy using a Neubauer haemocytometer; which is a slide with a grid accurately etched into the surface, and a precision of 99% was found.

At x20 magnification the section was saved as three images and was reconstructed in an image editing software programme[2] (Figure 2.2). Due to the age of the pilot foal the epidermal hoof width was greater than that anticipated for the full study where fetuses and foals aged up to four months would be sampled. It was therefore predicted that slides of fetal epidermal hoof wall would not need compounding and only older paediatric foals would require two images. It proved problematic to identify and therefore accurately classify all tubules into the three main types of tubules identified by Leach (1980). Although it was always possible to select an individual typical tubule, many had the characteristics of two types and deciding into which category to place them, would be highly subjective (Figure 2.3).

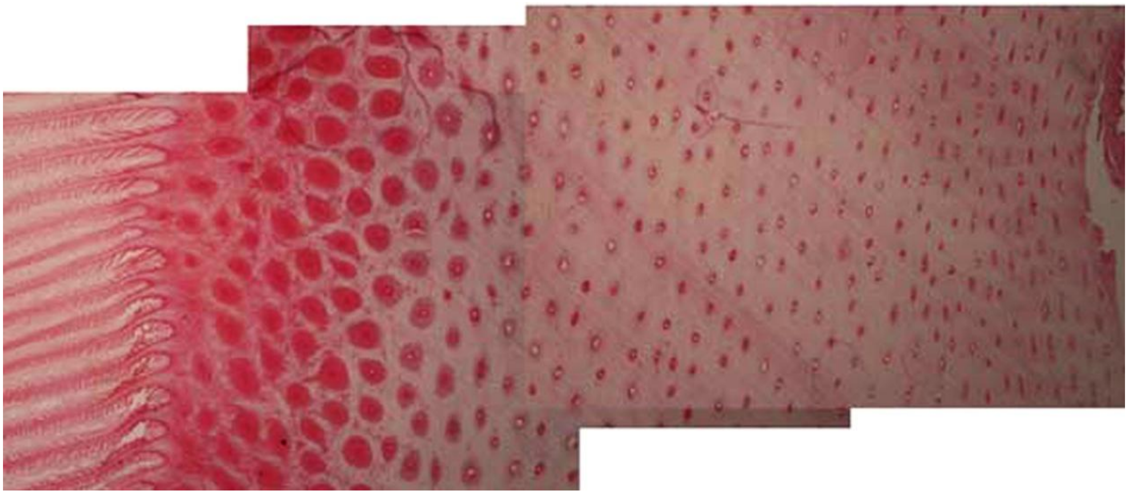


Figure 2.2: *Three separate microscopic images were matched and overlaid to reconstruct a compound image from lamellae (left) to abaxial wall (right).*

2.2.3: *Outcome*

Histological samples were produced to a standard which allowed analysis, but because of the subjective nature of identifying tubules into types, it was decided that data would record numbers of tubules and the overall density would be calculated by dividing tubule number by area. The area was created in

Photoshop as the width of the epidermis by one millimetre (Figure 2.3). Magnification of x20 proved to be sufficient for measurement requirements.

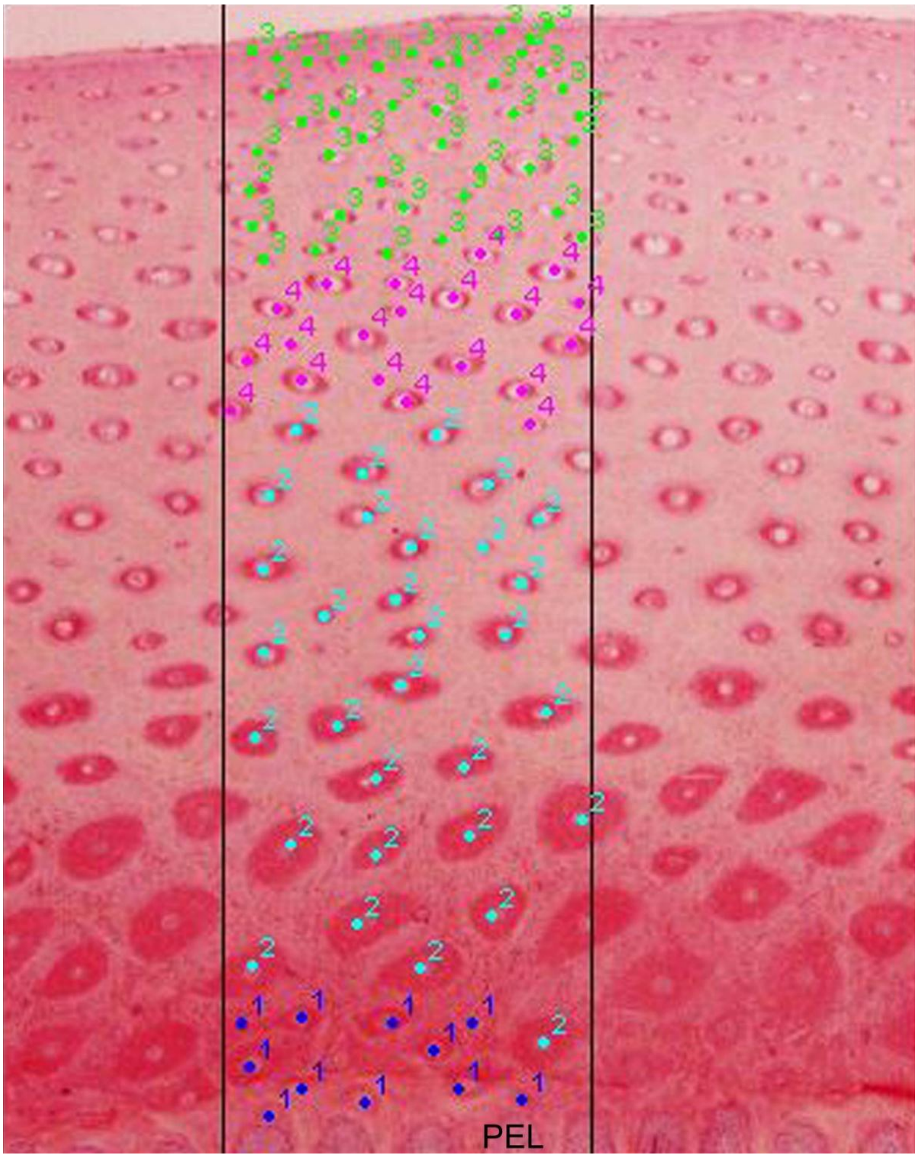


Figure 2.3: *The tubules in this transverse slide were differentiated using the Leach (1980) method; Type 1 tubules (dark blue, close to PEL), Type 2 (light blue, large and rounded), Type 3 (green, small and oval), Type 4 intermediate (purple, sharing the characteristics of 2 and 3),* PEL, *primary epidermal lamellae.*

2.3: Radiographic methodology

2.3.1: *Methods*

The front limbs of three foals on one stud farm were radiographed, taking dorsopalmar and lateromedial views. The usual radiographic procedures for safety were followed and the number of horse handlers and radiography operators determined. A qualified veterinary surgeon trained in radiography used a mobile radiography unit[3]. The problem of beam obliquity causing distortion of distances was overcome by placing a disc of known diameter in the plane of interest in the manner of Kulkani *et al* (2008). Various sizes were tried and the most suitable by cost and availability, was found to be a penny coin, which has a diameter of 20mm (Figure 2.4).

Prior to radiography, the midline sagittal centre (MSC) of the dorsum was established by marking the centre of the toe with an indelible black line taken through the middle of the frog to the point of toe. Using the marked toe, the radio-opaque disc was fixed to the dorsal hoof wall below the coronary band at the MSC, using adhesive tape. The disc was used to calibrate the radiographic image in order to measure the distal phalanx, hoof width and integument depth.

The sedated foals were stood upon two 50mm wooden blocks on a level floor with the metacarpals vertical. The beam was aimed at the centre of the foot for all radiographs and standard radiographic LM and dorsopalmar (DP) views taken (Butler *et al*, 2013). After each radiograph was taken its image was immediately viewed to ensure that it was of the required quality, with condyle alignment (Butler *et al*, 2013). If needed, adjustments were made to the beam angle and the stance of the foal and the radiograph was then retaken. The images were saved in Dicom format and viewed in Osirix[4] with each variable measured three times and saved in Excel[5]. The author was trained in Osirix[4] measurement procedures by the radiologist and recorded all measurements in Excel[5].

2.3.2: *Outcome*

The disc allowed calibration of all distances in the images. Statistical testing in Minitab 16[6] gave the mean deviation from actual; <0.1mm (SE 0.03mm). 30 measurements of an object of known dimension were tested for repeatability (Bland and Altman, 2010). Two points were outside the limits of agreement given; mean difference 0.01mm; upper limit of agreement, 0.04mm; lower limit of agreement , -0.03mm. 93.3% of data pairs were within the tolerances of the test. Measurements were therefore reported at 0.1mm minimum.

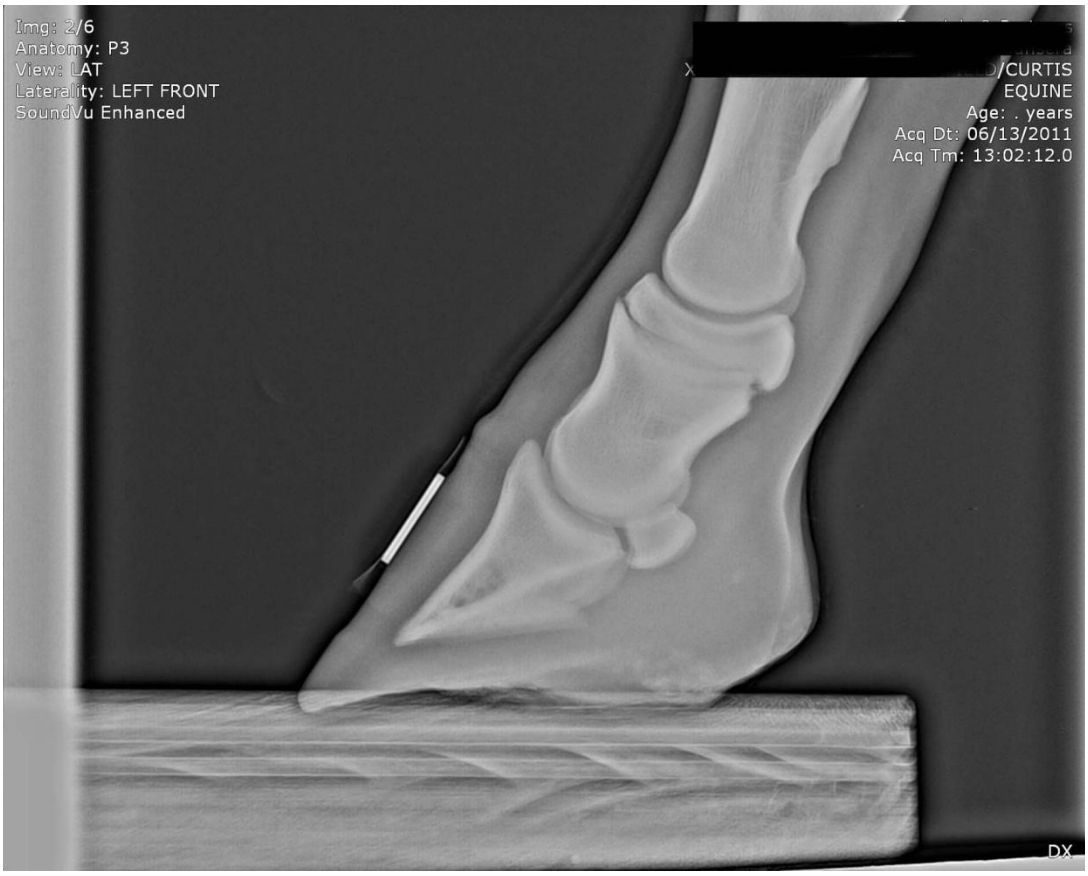


Figure 2.4: *A disc of 20mm diameter was placed on the dorsal wall to allow calibration of morphometrics.*

2.4: Hoof growth and compression methodology

A number of pilot studies to determine the most reliable and accurate methods for measuring hoof growth in foals were undertaken plus a method to test for plastic compression of horn was developed and appraised.

2.4.1: *Pilot study of twelve foals*

A pilot study of the left front of twelve foals situated on one farm, to determine the accuracy and repeatability of manual and photographic methods was carried out in one afternoon.

2.4.2: *Methods*

The author and an independent operator measured the hooves. The technique to be employed was explained and demonstrated to the second operator. The hoof was held lifted, drawn forward and a transparent ruler was used to measure the distance from the coronary hairline (CH) to the line in the hoof marking the day of birth, called here, the foal hoof crease (FHC). Each operator measured and recorded independently. Photometry was carried out solely by the author using an iPhone[6] camera, with a calibration disc of 12.7mm attached adjacent to the area of measurement. The hoof was lifted and held forward by the second operator while the author photographed it. The camera was held at approximately 30mm distance and aimed at 90° to the dorsal hoof wall angle (DHWA). Three photographs were taken and the most suitable downloaded to a software programme[9] designed for measuring hooves. The data were tabulated in Excel[5], and analysed in Minitab 16[6] for correlation and ANOVA.

2.4.3: *Outcome*

The results showed that measurements to a precision of 0.5 mm, acquired by either method could be achieved and there was no difference amongst the means of the data; P=0.76. Thirty measurements of the 12.7mm calibration disc were tested for repeatability (Bland and Altman, 2010). One point was outside the limits of agreement given; mean difference -0.06mm; lower limit of agreement, -0.04mm. 96.6% of data pairs were within the tolerances of the test. Measurements were therefore reported at 0.1mm minimum.

The subsequent hoof growth rates (HGR) derived from this data also did not differ; P=0.28 (Figure 2.5). Foal 1 in this study was the youngest and proved the most difficult to measure as the FHC was not as easily identifiable at this age. The manual method of morphometric collection was simpler but all later studies used photometry as the images could be stored and re-measured.

2.5: Pilot study of three foals

2.5.1: *Methods*

A second pilot study of three foals used the photographic method described above over a period of three months to record hoof growth. Dependent upon the availability of the foals, they were measured six, eight, and nine times. Using this method on young foals proved accurate in giving hoof growth and HGR. There was a positive correlation of hoof growth to time; R^2=0.98, P=0.001 (Figure 2.6). When the derived hoof growth rates of each measurement were tested for correlation with age there was a decline, R^2= -0.73, P=0.001 (Figure 2.7).

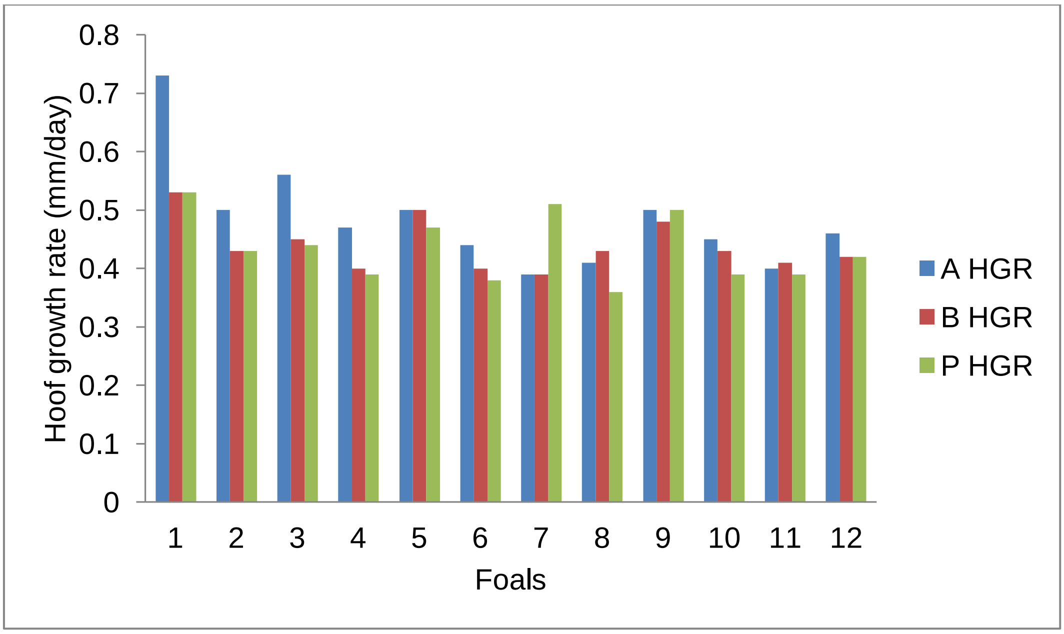


Figure 2.5: *The hoof growth rates (mm/day) of 12 foals acquired by manual and photometric methods;* A*) the manual measurements of an apprentice farrier;* B*) author's manual measurements;* P*) photographic images quantified in measurement software.*

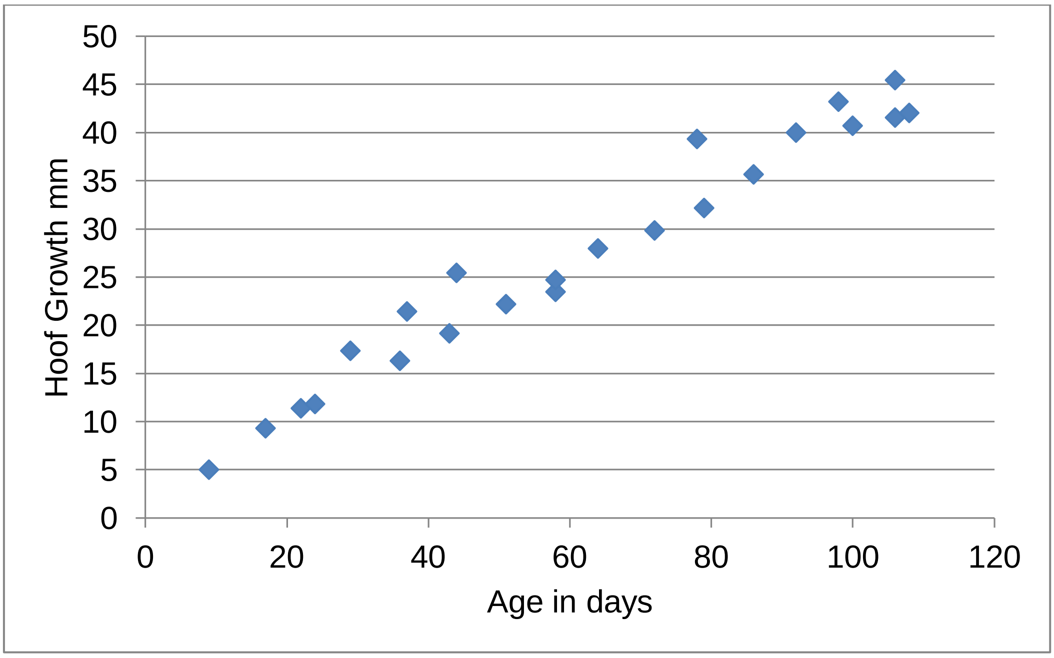


Figure 2.6: *Hoof growth to age y = 0.3788x + 3.8828, (n=3).*

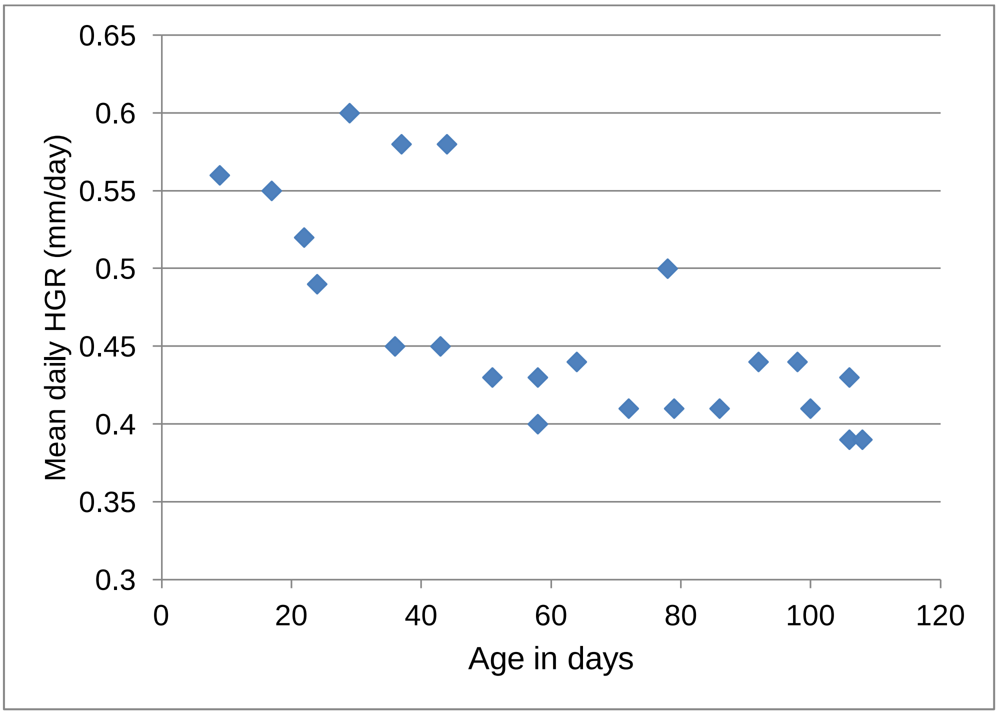


Figure 2.7: *Mean daily HGR to age y = -0.0016x + 0.5618 (n=3).*

2.5.2: *Outcome*

The results appeared to confirm the accuracy of using this method and drew attention to the speed of hoof growth at this age. The line marking birth disappeared at approximately four months at the dorsum and at the heels was gone by two months of age. It was therefore decided that a method of marking the hoof was required that was not dependent upon the FHC.

2.6: Marking hooves

Previous authors have marked hooves for hoof growth studies by rasping a line across the hoof wall or burning a mark into the wall (Butler and Hintz, 1977; Glade and Salzman, 1985; Reilly *et al*, 1998[b], Faramarzi *et al*, 2009). These methods were crude and carried risks which were considered unacceptable for foals. It was decided to develop a safer and more suitable method of producing marks which would remain until grown down to the distal bearing border where they would be worn away or removed by routine trimming.

2.6.1: *Methods*

Three foals on one stud farm were used to develop a method of measuring hoof growth and establishing whether vertical plastic deformation could be measured. Before live marking of foals, cadaver specimens were examined to ensure the safe depth and position of holes. Two foals had the front left dorsum marked with a hole drilled at 4mm diameter by 2mm depth, filled with hoof repair acrylic[8]. An additional foal had two holes drilled into the wall approximately 20mm apart in vertical alignment (Figure 2.8). Various sizes, shapes and coloured calendar stickers were used to attach calibration to the hoof. The images were measured using the same software package[9] used earlier. The foals were photographed at approximate monthly periods for four months and at the end of this time the marks were still clear.

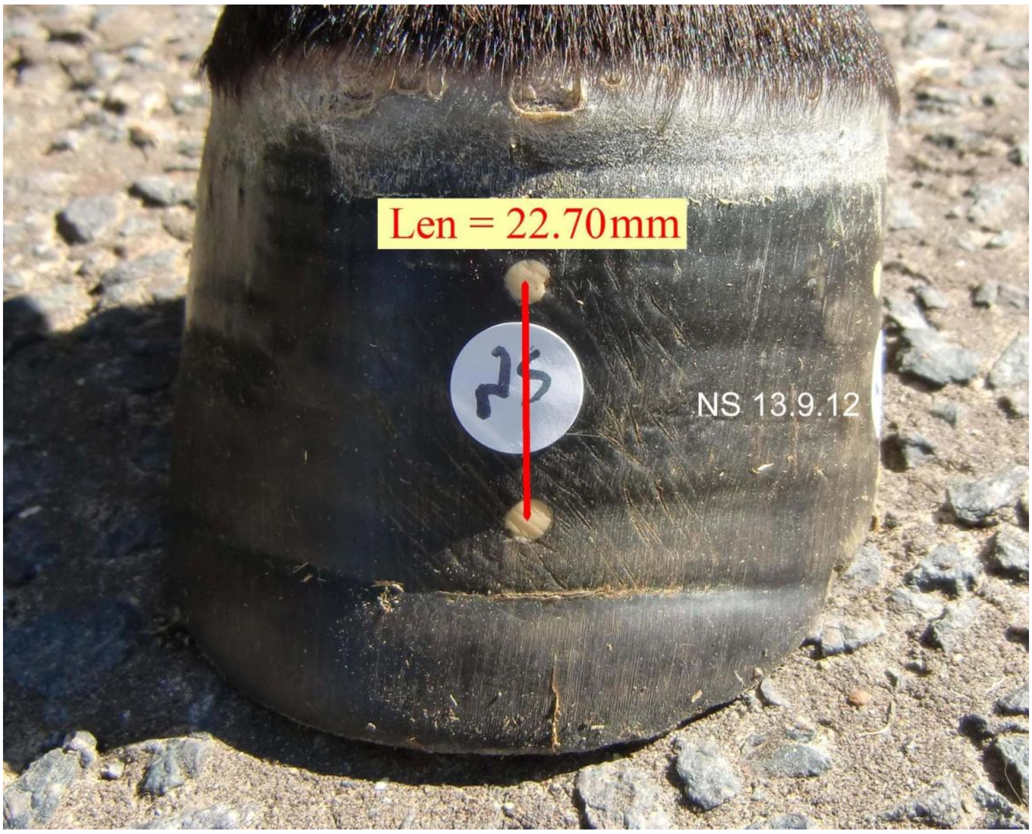


Figure 2.8: *In the pilot study the holes, filled with acrylic, were 4mm in diameter and over 20mm apart. Here a 12.7mm diameter calendar sticker is attached with the foal's initials.*

2.6.2: *Outcome*

The distance between the two marks on one foal appeared to decrease and this stimulated the hypothesis that hoof horn compressed during descent after generation (Figure 2.9). It was decided that the diameter of the holes should be 2mm and approximately 12mm apart as these changes would improve accuracy. Additionally this would mean that the distal mark was further from the bearing border of the hoof, allowing more time before it disappeared. Circular wall calendar stickers of 12.7mm diameter, coloured either white, blue or green proved to be ideal in size and did not produce a blurred edge in images as some colours did. It was decided that marking the coronary hairline (CH) in red would improve location in images.

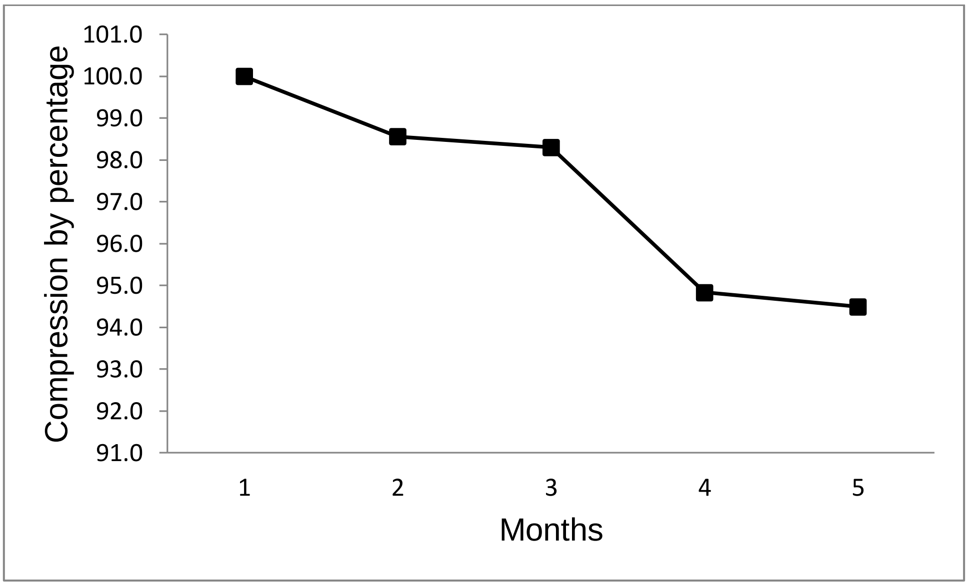


Figure 2.9: *Pilot compression measurement; the original distance between marks (1) was given a value of 100 and the four subsequent monthly measurements (2-5) were compared as a percentage (n=1).*

2.7: Weight-bearing, solar loading and hoof growth

2.7.1: *Methods*

The position of 2x2mm marks were tested on a cadaveric hoof capsule, age one month and later a live foal. It was decided that the heel distance meant that the distal mark would disappear in one month, not allowing time for sufficient data to be collected, and therefore the marks would be positioned at the dorsum and the widest part of the foot (quarters) approximately 15mm below the coronary hairline and again approximately 12mm distal of this mark in alignment with the horn tubules. The use of a pressure mat was first viewed at the Veterinary Faculty, University of Utrecht, where it was used in conjunction with a force plate and video cameras. The author also had the opportunity to view miniature pigs trotting over a pressure mat and this gave an indication of the resolution of data that it was possible to acquire. In the UK a pressure mat[10] was tested with a foal of 3 weeks age to assess safety procedures and to ensure that sufficient cells were covered by the small hooves of young foals, to produce meaningful data (Figure 2.10). It was decided that walking

Thoroughbred foals over a mat was neither viable nor safe and that useful data could be collected by quasi-static methods, with both front feet on a pressure mat (Hood *et al*, 2001).

The technician operating the software[10] decided upon which 20 second data recording showed the least sway and selected frame 500 for analysis. Calculating foot orientation, axial position and quadrant values was carried out by the author (Figures 2.10 and 2.11).

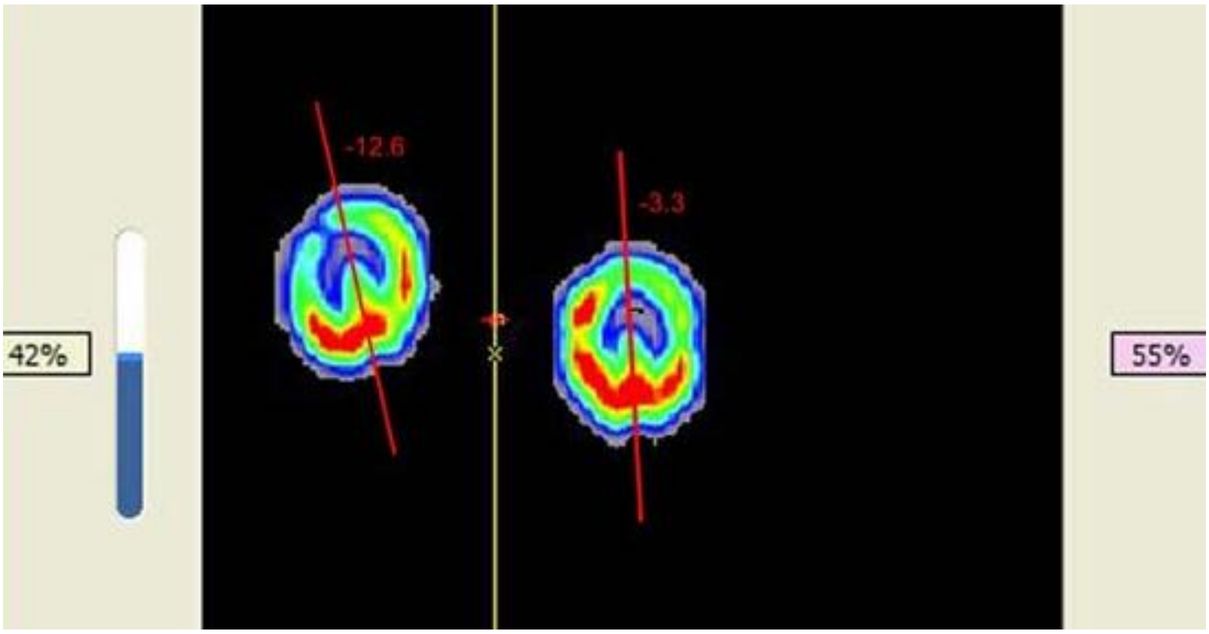
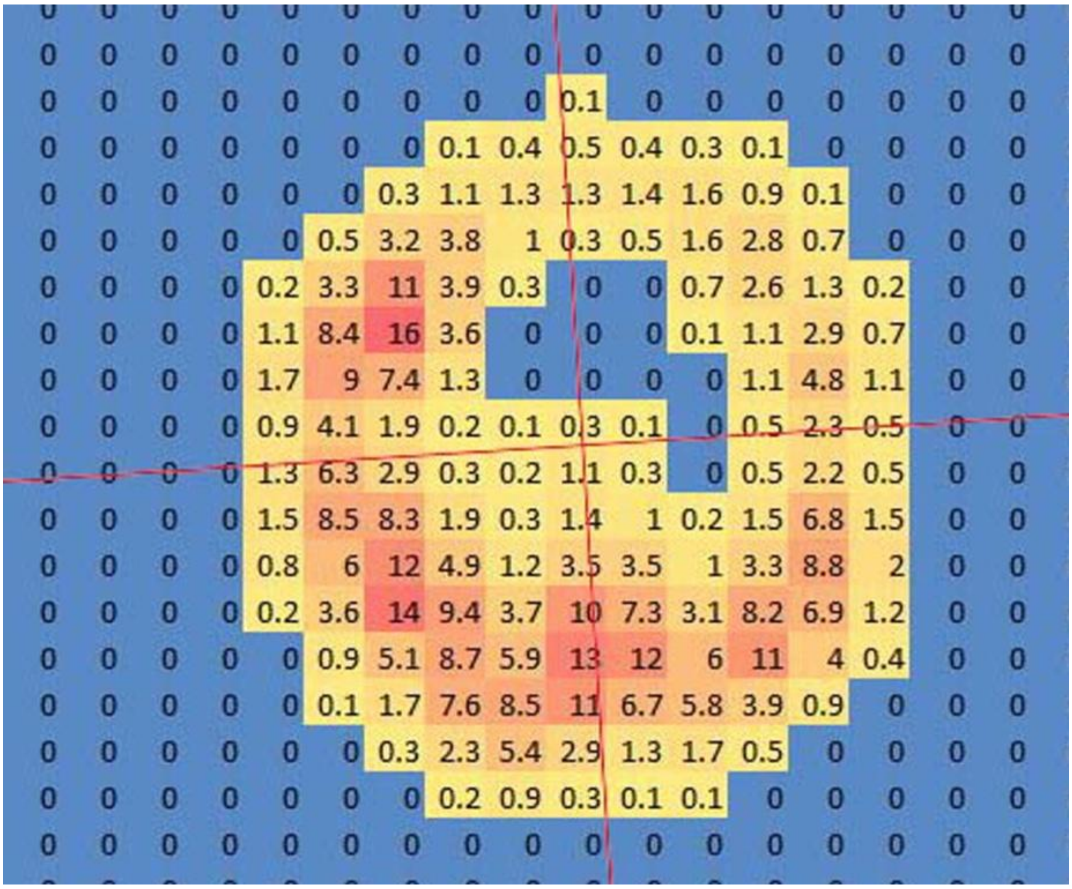


Figure 2.10: *A captured frame from the video of a three week old foal standing on a pressure mat. The percentage of weight-bearing between left and right is shown; the orientation (-12.6° and -3.3°) had been established using an image editing programme[2].*

There were a number of problems encountered with standing both front feet upon a pressure mat: 1) the centre of pressure would be found between the two feet; 2) the feet would not be orientated on the x-y axes of the mat; 3) foals rarely stand with their weight equally distributed between front limbs (Figure 2.10). Additionally, with the relatively small area covered by the foot, the resolution or number of cells could be too small to produce meaningful data to differentiate between regions of the solar foot.

These problems were overcome by capturing an image of the pressure mat data to establish the dorsopalmar axis of each foot in Photoshop 7.0[2] (Figure

2.10). A xy axis was created and rotated on the image of the pressure mat spreadsheet data, with the axis centred at the geometric midpoint of the data to isolate the cells into quadrants. Where the axes crossed cells then the value of the cell was equally shared between adjoining quadrants (Figure 2.11).



0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0.1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0.1	0.4	0.5	0.4	0.3	0.1	0	0	0	0
0	0	0	0	0	0	0.3	1.1	1.3	1.3	1.4	1.6	0.9	0.1	0	0	0
0	0	0	0	0	0.5	3.2	3.8	1	0.3	0.5	1.6	2.8	0.7	0	0	0
0	0	0	0	0.2	3.3	11	3.9	0.3	0	0	0.7	2.6	1.3	0.2	0	0
0	0	0	0	1.1	8.4	16	3.6	0	0	0	0.1	1.1	2.9	0.7	0	0
0	0	0	0	1.7	9	7.4	1.3	0	0	0	0	1.1	4.8	1.1	0	0
0	0	0	0	0.9	4.1	1.9	0.2	0.1	0.3	0.1	0	0.5	2.3	0.5	0	0
0	0	0	0	1.3	6.3	2.9	0.3	0.2	1.1	0.3	0	0.5	2.2	0.5	0	0
0	0	0	0	1.5	8.5	8.3	1.9	0.3	1.4	1	0.2	1.5	6.8	1.5	0	0
0	0	0	0	0.8	6	12	4.9	1.2	3.5	3.5	1	3.3	8.8	2	0	0
0	0	0	0	0.2	3.6	14	9.4	3.7	10	7.3	3.1	8.2	6.9	1.2	0	0
0	0	0	0	0	0.9	5.1	8.7	5.9	13	12	6	11	4	0.4	0	0
0	0	0	0	0	0.1	1.7	7.6	8.5	11	6.7	5.8	3.9	0.9	0	0	0
0	0	0	0	0	0	0.3	2.3	5.4	2.9	1.3	1.7	0.5	0	0	0	0
0	0	0	0	0	0	0	0.2	0.9	0.3	0.1	0.1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2.11: *Numerical data of the foot shown in Figure 2.10, giving values of force with an overlaid xy axis centred and rotated -3.6° to divide the weight-bearing into quadrants; by summing the quadrants, medial, lateral, dorsal and caudal, regional totals were produced.*

2.7.2: *Outcome*

On the atypically small foot illustrated (Figure 2.11) there were 143 cells within the weight-bearing area, 11 of which had a zero value. The resolution produced was therefore considered sufficient to produce data for the study design.

Chapter 3: The structure of the epidermis in fetuses and foals

3.1: Introduction

This chapter quantifies the hoof wall epidermal thickness, size, and density of horn tubules in late fetuses and paediatric foals. The hoof wall is a highly adapted integument with a tubular structure which is heterogeneous axioabaxially and bears weight immediately *post partum*. The hoof wall must therefore be, to some extent, prepared for weight-bearing. It is not known whether the hoof wall is pre-adapted to weight-bear or whether it responds *post partum*.

In the mature horse the epidermal hoof wall varies in thickness around the hoof capsule; it is thickest at the dorsum while the medial epidermis is thicker than the lateral epidermis (Lancaster *et al*, 2013). The structure of the hoof wall has been documented in mature horses, showing differing horn tubule densities at the dorsum, medial, and lateral epidermal regions; while there appears to be no data for foals (Leach, 1980; Lancaster *et al*, 2013). It is not known if the hoof wall thickens during maturation by increasing tubular diameter, adding tubules, reducing tubular density, or a combination of these.

3.1.1: *Study aims:*

The aims of this study were to document epidermal hoof wall increase in thickness, to measure tubular diameter and density in fetuses and paediatric foals. The objectives were; 1) to collect hoof tissue samples from cadaver fetuses and young foals; 2) to measure tubular diameter, density and hoof wall thickness in the medial, dorsal, and lateral epidermis; 3) to compare tubular diameter and density, and hoof wall thickness by age and location.

3.1.2: *Hypotheses:*

The hypotheses were; 1) the medial epidermal hoof wall would be wider than the lateral hoof wall and narrower than the dorsal hoof wall; 2) tubular diameter and density would be related to age; 3) and tubular diameter and density would differ around the hoof wall.

3.2: Methods

3.2.1: *Ethical agreement:* The owner of the foals and fetuses understood and agreed to this study. Ethical approval was given by the University of Central Lancashire Animal Projects Committee (RE/12/06/SH).

3.2.2: *Data collection and preparation*

The front legs of 15 Thoroughbred cadaver fetuses and foals were disarticulated at the fetlock and frozen within two hours of death. The causes of death were unrelated to this study; none of the subjects were euthanised and all had healthy legs and hooves. All legs were labelled with age; if fetuses, their dam's last service date, date of collection and foals date of birth and death. On the same day all the left front digits were removed from the freezer, cleaned and marked with an indelible pen at the widest solar dimension and centre of dorsum. Sections were cut midway between the coronary band and bearing border, parallel to the coronary band and at 90° to the plane of the hoof wall (Figures 2.1 and 3.1). Histological preparation of slides followed the method described in section 2.2.2, Chapter 2. Any unusable slides were discarded and new ones cut; any hoof that did not provide all three medial, dorsal, and lateral samples was rejected. Images from the slides were orientated so that the lamellae were at the bottom and the abaxial wall at the top. These images were viewed at x20 and x40 magnification[11], recorded with an embedded calibration scale and saved as lossless Jpeg files.

3.2.3: *Measurement of data*

Using Photoshop 7.0[2], the images had two parallel lines added with a distance of one mm between. Where necessary, images were compounded to create a full width image, as described in section 2.2.2, Chapter 2.The images were measured using a software programme specifically designed for cell counting[12] (Figure 3.2). The epidermis thickness and the three largest tubules were measured three times and the means recorded. All horn tubules were counted within the parallel lines, only including tubules where the whole of the medulla was visible. Data were stored in Excel[5] and statistical analysis was completed using Minitab 16[6]. Density was calculated by dividing the number of tubules by the area measured.

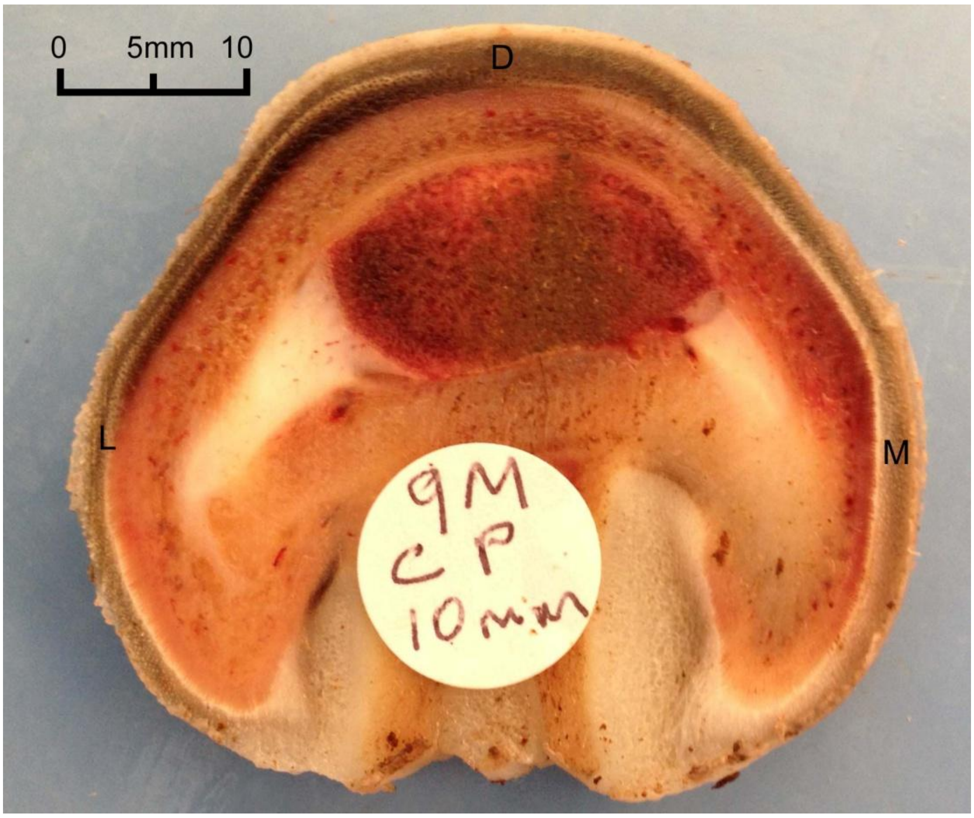


Figure 3.1: *A mid-wall transverse section through the foot, parallel to the coronary hairline, approximately 10mm thick; see Figure 2.1 for orientation. Samples of the dermoepidermis were taken from three sites; L) lateral, D) dorsal, M) medial. The label denotes age of fetus (9 months), initials of dam's name, and thickness of cut.*

3.2.4: *Statistical analysis*

The data were recorded as three variables in dorsal, lateral, and medial regions. The variables were; 1) epidermis thickness in millimetres (mm), from this measurement area was calculated in mm^2; 2) largest horn tubule diameter; 3) number of horn tubules in the area, from this density by mm^2 was calculated. The age of the foals was recorded in days and the age of the fetuses was calculated from the last service date and recorded as a negative age in days from the due date of birth. Data were classified into four categories; fetus, neonate, neonate to 2 months, 2 months plus. All data were tested in Minitab 16[6] for normal distribution using an Anderson-Darling test which was set at $P>0.05$. Initial exploratory principal component analysis (PCA) of all data was used to highlight patterns in the data. Further analysis compared the three regions using ANOVA for epidermis thickness, horn tubule diameter and density. Pearson correlation compared days of age, epidermis thickness, tubule diameter, and density.

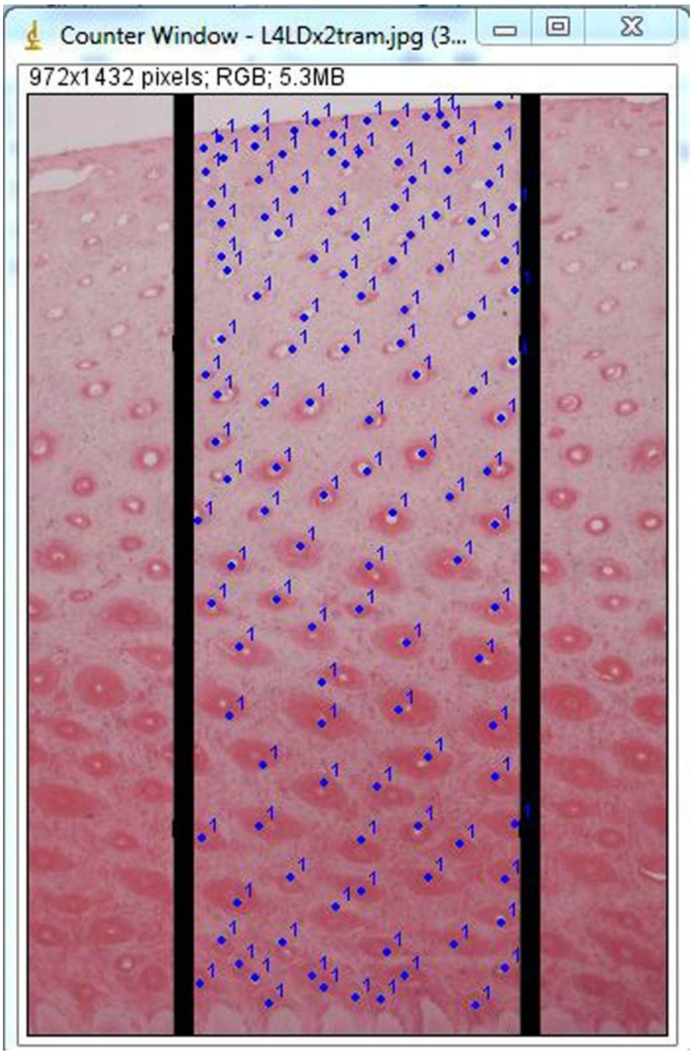


Figure 3.2: *Tubules were counted using ImageJ[12] only where the medulla was within the area.*

3.3: Results

3.3.1: *Principal Component Analysis (PCA)*

The variables, tubule density and epidermal width were spread along the PC1 x axis and explained 56.6%of the variation. Separation along the y axis of PC2 of tubule diameter and age explained 19.9% of the variation (Figure 3.3). The age groups had different cluster patterns; with aging the variables were less influenced by tubule density and diameter and more by epidermis width and age.

3.3.2: *Epidermis thickness (mm±SD)*

The mean epidermis thicknesses were: medial, 2.41±0.44mm; dorsal, 3.31±0.95mm; lateral, 2.36±0.58mm. Differences among the means were significant, P=0.013. The means of the medial and lateral epidermis thickness at the quarters did not differ, 0.049±0.39mm, P=0.63; and were significantly less than the dorsal epidermis; P=0.001. There was a positive correlation of epidermis thickness to age; *r*=0.41, P=0.005.

3.3.3: *Horn tubule diameter (mm±SD)*

The mean of the largest tubule diameters were: medial, 0.12±0.02mm; dorsal, 0.16±0.03mm; lateral epidermis, 0.13±0.03mm. Differences among the means were significant, P=0.002. The means of the medial and lateral tubule diameter at the quarters did not differ, 0.006±0.02mm, P=0.231, and were significantly less than the dorsal tubule diameter; 0.033mm, P=0.005. There was a positive correlation of horn tubule diameter to age; *r*=0.31, P=0.04. Horn tubule number increased with age; *r*=0.30, P=0.048.

3.3.4: *Horn tubule density (/mm²±SD)*

The mean horn tubule densities were: medial, 42.1±5.2/mm^2; dorsal, 36.3±5.5/mm^2; lateral, 42.2±4.9/mm^2. Differences among the means were significant, P=0.008. The means of the medial and lateral tubule densities were not significantly different; -0.06mm/^{2}. The density of the horn tubules at the dorsum was significantly less than at the quarters; -5.8/mm^2. There was a negative correlation of horn tubule density to age; *r* = -0.30, P=0.043.

3.3.5: *Correlations between variables*

There was a significant negative relationship between epidermis thickness and density; *r*=-0.40, P=0.007 and a significant positive relationship between epidermis thickness and largest horn tubule diameter, *r*=0.74, P=0.001.

3.3.6: *Qualitative observations*

The transverse sections of epidermis showed the characteristics recorded by previous authors of mature hoof (Leach, 1980; Lancaster, 2013). The pattern of tubules was Type 1 adjacent to the lamellae; Type 2, larger than Type 1, extending 50-60% into the *stratum medium* towards the abaxial surface and merging with Type 3 which became progressively more oval and small at the abaxial surface. Sixteen (37%) of the slides gave the impression of a relationship between the lamellae tips and tubules which appeared to flow away from them into the *stratum medium*, while the remainder did not (Figures 1.7 and 3.4).

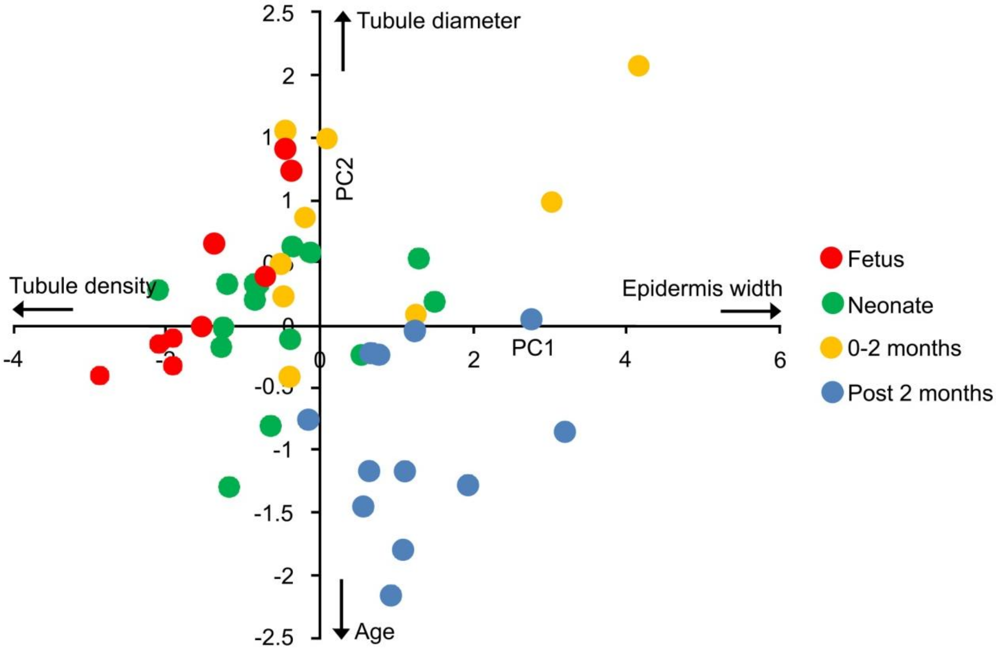


Figure 3.3: *Principal component analysis; along axis PC1, epidermis width and tubule density show the greatest positive (Epidermis width) and negative (Tubule density) effect on each other. When graph points were coloured according to categories; fetus (n=3), neonate (n=5), birth to two months (n=3), and post two months (n=4), the separation occurred along PC2.*

3.4: Summary of findings

This chapter investigated the widening epidermal hoof wall in fetuses and foals. The results confirmed that with aging the epidermis thickened and horn tubules increased in diameter, also as the epidermis widened the tubular density decreased. The first hypothesis; that the medial epidermis would be wider than the lateral epidermis and narrower than the dorsal, was rejected. The second hypothesis; that tubular diameter and density is related to age, confirmed. The third hypothesis; that tubular diameter and density would differ around the hoof wall, was rejected. Tubular diameter did not differ between the quarters but was greater at the dorsum and the density did not significantly differ in the three regions. A discussion of these findings is found in Chapter 7.

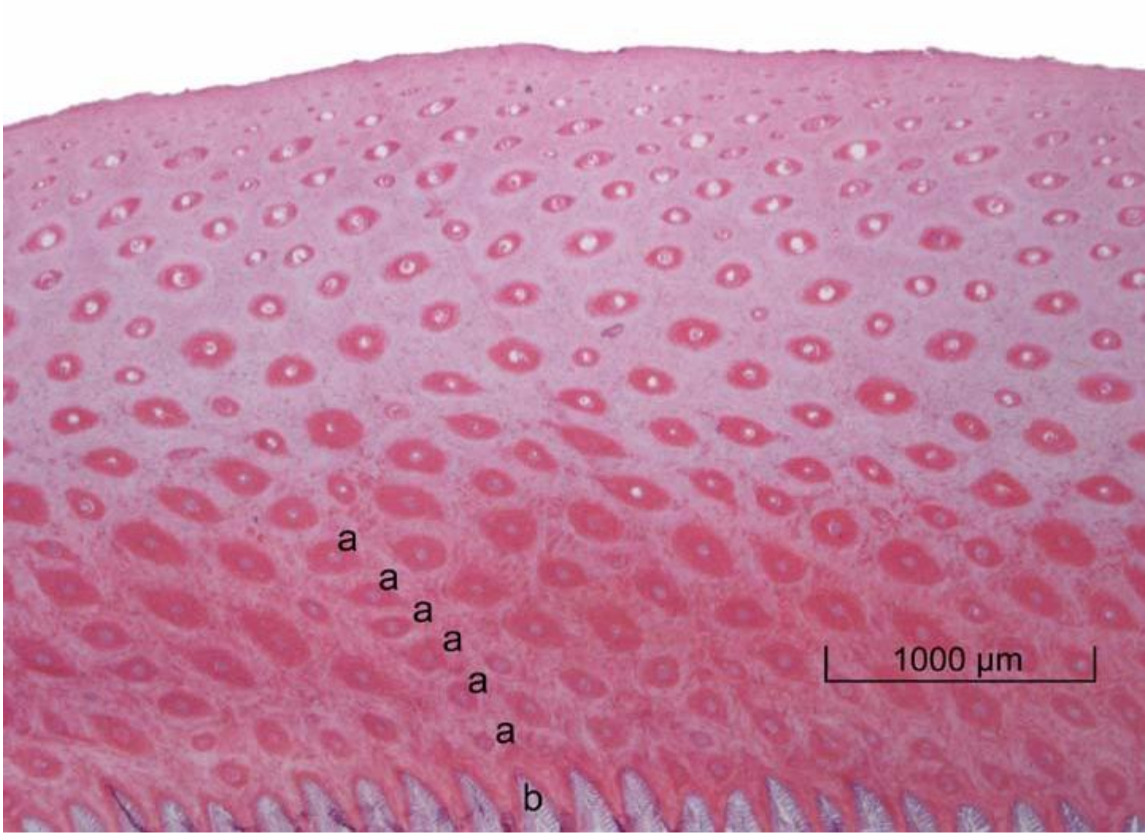


Figure 3.4: *The horn tubules (a), from the hoof wall of a neonate, appear to be flowing from the tips of the lamellae (b) which can be seen at the bottom of the slide.*

Chapter 4: Radiographic angles of the digit and solar hoof morphometrics in Thoroughbred foals

4.1: Introduction

This chapter considers the relationships between external angles of the digit, solar hoof morphometrics and skeletal angles, in a cohort of developing foals. The outer appearance or conformation of the horse is an important factor in breeding and Thoroughbred stud farms focus on it at an early age (Mawdsley *et al*, 1996). Poor hoof angle and hoof pastern alignment has been linked to lameness in mature horses and it is not known if these conformational weaknesses are congenital or acquired. In a study of distal phalanx fractures in 17 Warmblood foals no association was found with either dorsal hoof wall angle (DHWA) or hoof pastern axis (HPA) (Bhatnagar *et al,* 2010). The hoof wall integument and distal phalanx are parallel in healthy mature horses but not in foals and this lack of parallelism has not been explained (Kroenkenstoel, 2006). The correlation of uneven hoof angles to disparity of hoof widths found in mature horses has not been studied in foals (Mawdsley *et al*, 1996; Wilson *et al*, 2009). The HPA is an established conformational assessment and an aligned HPA has been considered beneficial to soundness and used as a guide to farriery in mature horses (van Weeren and Crevier-Denoir, 2006; Willemen *et al*, 1999). DHWA has been shown to decline from weanling to three years old but DHWA has not been measured from paediatric to weanling (Anderson and McIlwraith, 2004).

4.1.1: *Study aims*

The aims of the study were 1) to document the morphometrics of the hoof capsule and phalanges in Thoroughbred foals, and 2) to quantify changes in conformation and morphometrics that occur due to growth and development in the first year of life.

4.1.2: *Hypotheses*

It was hypothesised that: 1) the phalangeal axis and HPA would not be aligned in foals, 2) there would an association between front hoof width disparity and DHWA disparity, and 3) DHWA would decline with age.

4.2: Materials and method

4.2.1: *Selection of foals*

Twenty two TB foals were selected with the following criteria; they were born in the same year at one of three stud farms, their ages were within a four month range, they shared the same veterinary practice and farrier (the author). All foals and their dams were controlled by experienced handlers and monitored throughout the measurement procedures to ensure that no undue stress was caused to the mare or foal.

4.2.2: *Ethical agreement*

The stud farms' managements understood and agreed to the study. Ethical approval was given by Myerscough College, reference 5/JR/11/AN/75.

4.2.3: *Morphometric procedure*

All foals were radiographed and their hooves measured in the month of June, between the 13th and 16th and in November, all on the 11th. These months were selected because by mid-June all foals would be at least one month old and after November some foals would be sold and therefore be unavailable for study. All of the foals' hooves were trimmed by the author, none less than two weeks prior to data collection. The solar plane of the hoof wall was trimmed to approximately 3mm from the cleaned sole at 90° to the long axis of the cannon, aiming for an aligned HPA. The distal border was rounded to an approximate 3mm radius. No rasping of the outer wall took place.

There are few fixed points on the foot and the hoof capsule is not geometric but irregular and parts merge into each other with ill-defined borders. Curtis (1999) and Beveridge (2002) used three dimensions to measure the solar hoof; width at the widest part of the foot, length from toe to point of buttress through the frog apex; and heel width between the points of the buttresses. These measurements were used in this study (Figure 4.1). Each hoof was measured three times to an accuracy of 0.5mm and the mean for each dimension calculated.

4.2.4: Radiographic procedure:

A mobile radiography unit[3] was used by the same radiologist for all foals. The radiographic methods are described in 2.3.1, Chapter 2 were repeated for this study.

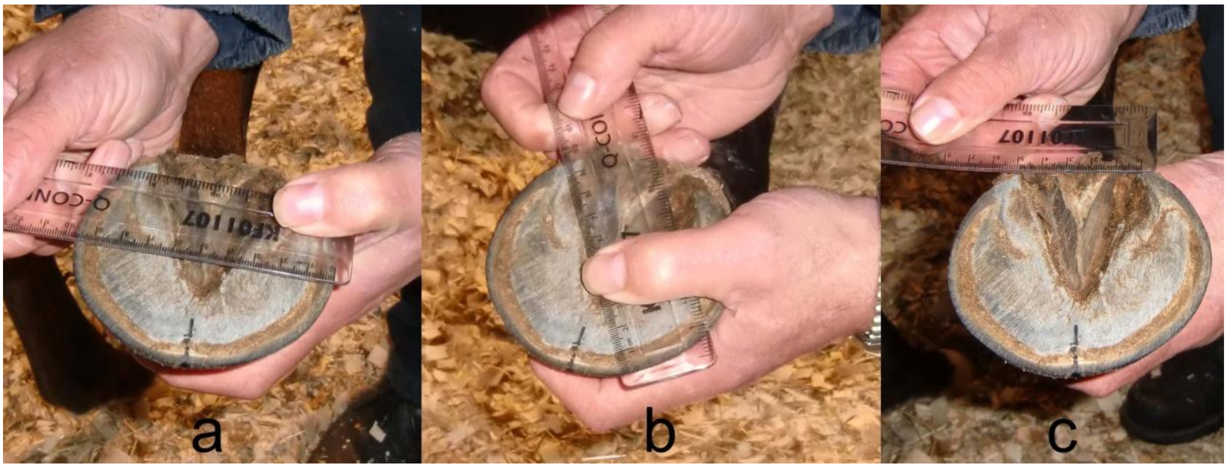


Figure 4.1: *The three solar measurements were; a) hoof width at the widest point, b) hoof length from point of buttress to toe via the frog apex, c) heel width between the points of buttress.*

Radiographic images were viewed on screen and measured using Osirix[3]. Measurement units were millimetres (mm) and angles in degrees (°). The LM views were used for all angle measurements and also for the integument depth (Figure 4.2). The proximal integument depth (PID) was measured from the centre of the concave curvature of the distal phalanx (PIII) to the abaxial extent of the dorsal wall at 90º to the DHWA. The distal integument depth (DID) was measured from the distal tip of PIII to the abaxial extent of the dorsal wall at 90º to the DHWA. Width of the distal phalanx and hoof capsule were measured at the widest dimension on the DP view after calibration using the disc attached to the DHW (Figure 4.3). Each measurement was taken three times from all images and transferred into an Excel[4] spread sheet where the mean was pooled.

4.2.5: *Defining hoof pastern axis and phalangeal axis*

The HPA was defined as the DHWA minus the pastern angle. This study employed the same method as Collins *et al* (2011) using a line through the centre of the pastern to determine its axis (Figure 4.4a). The phalangeal axis was defined as the DPA minus the sum of the angles of the proximal phalanx and middle phalanx, an axis drawn from the centre of the proximal joint surface of the proximal phalanx to the centre of the distal joint surface of the middle phalanx (Figure 4.4b).

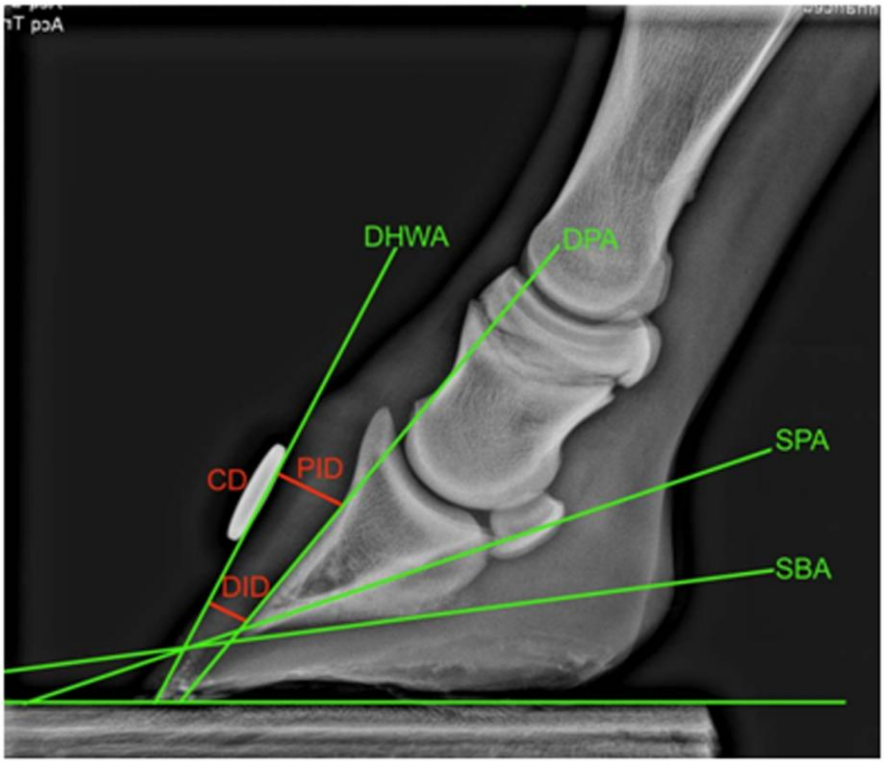


Figure 4.2: *A left front lateromedial radiographic image with angles in green and distance measurements in red; DHWA, dorsal hoof wall angle; DPA, dorsal parietal angle; SPA, solar parietal angle; SBA, solar border angle; PID, proximal integument depth; DID, distal integument depth; CD, disc used to calibrate PID and DID.*

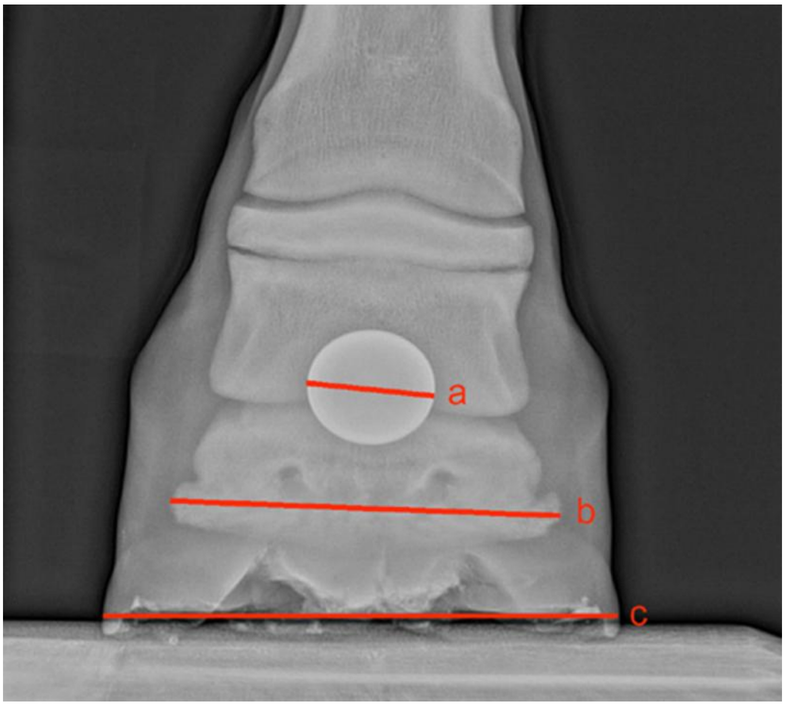


Figure 4.3*: A dorsopalmar radiographic image; a) the disc calibrated to 20mm across its widest dimension, b)* the distal phalanx *width, c) hoof width.*

4.2.6: *Statistical analysis*

All data were analysed using Minitab 16[6] and tested for normal distribution using an Anderson–Darling test. To document the morphometrics of the hoof capsule and phalanges summary statistics for each variable were calculated and the relationship between variables investigated using a Pearson correlation matrix. To quantify changes due to growth a paired *t*-test was used to compare June and November data. A paired samples *t*-test was used to compare the alignment of the phalangeal axis and HPA in individual limbs for June and November separately. Hoof widths of individuals LF and RF were compared using a paired *t*-test to investigate the disparity between feet. Where data were found to be non-normal, the equivalent non-parametric test was used (Wilcoxon matched pair test). The relationship between LF to RF hoof width differences and LF to RF DHWA differences was compared using Pearson correlation.

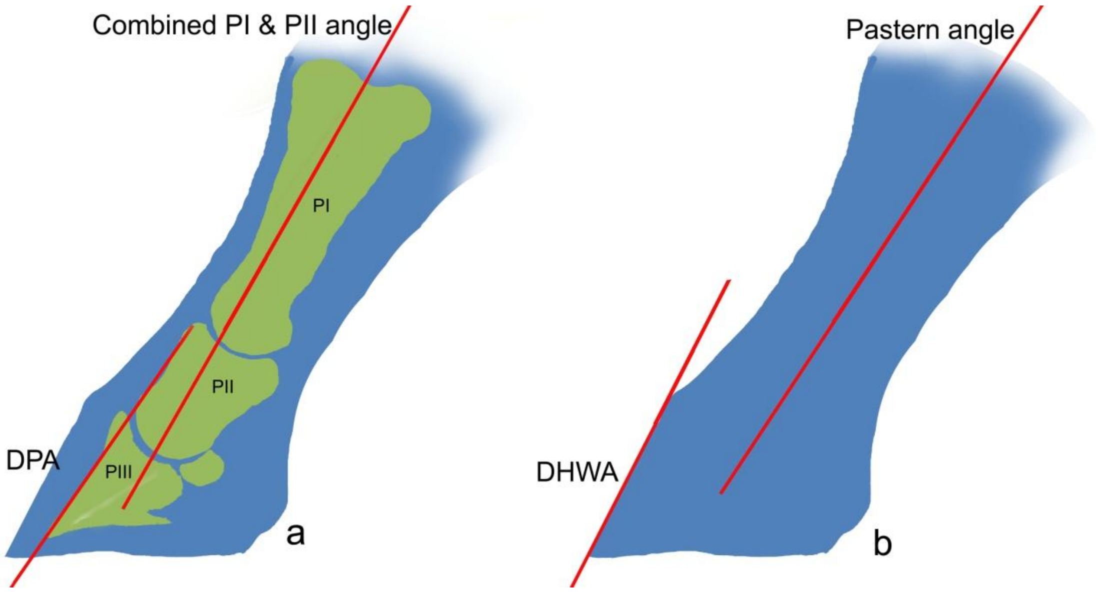


Figure 4.4: *a, the phalangeal axis was defined as the dorsal parietal angle (DPA) minus the combined angles of the proximal (PI) and middle (PII) phalanges; b, the hoof pastern axis was defined as the DHWA minus the pastern angle.*

4.3: Results

The foals' ages ranged from 33-145 days (±35) in June and 180-296 days (±35) in November. The radiographic and solar morphometrics of the TB foals are shown in Table 4.1 and radiographic angles are shown in Table 4.2. All these data had a normal distribution apart from phalangeal axes. The correlations between variables are shown in Tables 4.3 and 4.4. All data were shown as mean (±SD). Repeatability of the metrics derived from Osirix[4] were tested; mean deviation from actual, <0.1mm (SE 0.03mm). The intra-horse metrics showed low heteroscedasity; where the percentage coefficient of variation (across the variables) for June ranged from 0.1-1.4%, November 0.01-1%.

4.3.1: *Dorsal hoof wall angle, hoof pastern angle, phalangeal axis (°±SD)*

The combined left and right DHWA was 59.4°±3.7° in June and 53.5°±2.8° in November (Figure 4.5). The phalangeal axis in June and November was typically negative; mean for June, -11.0º±7.4°; mean for November, -8.3°±4.4°. The HPA in June and November was typically positive; mean for June, 6.1°±2.1°; mean for November, 2.5°±1.6°. The HPAs and phalangeal axes were significantly different; June, -17.1°±7.7°; November, -10.8°±4.8°, P=0.001.

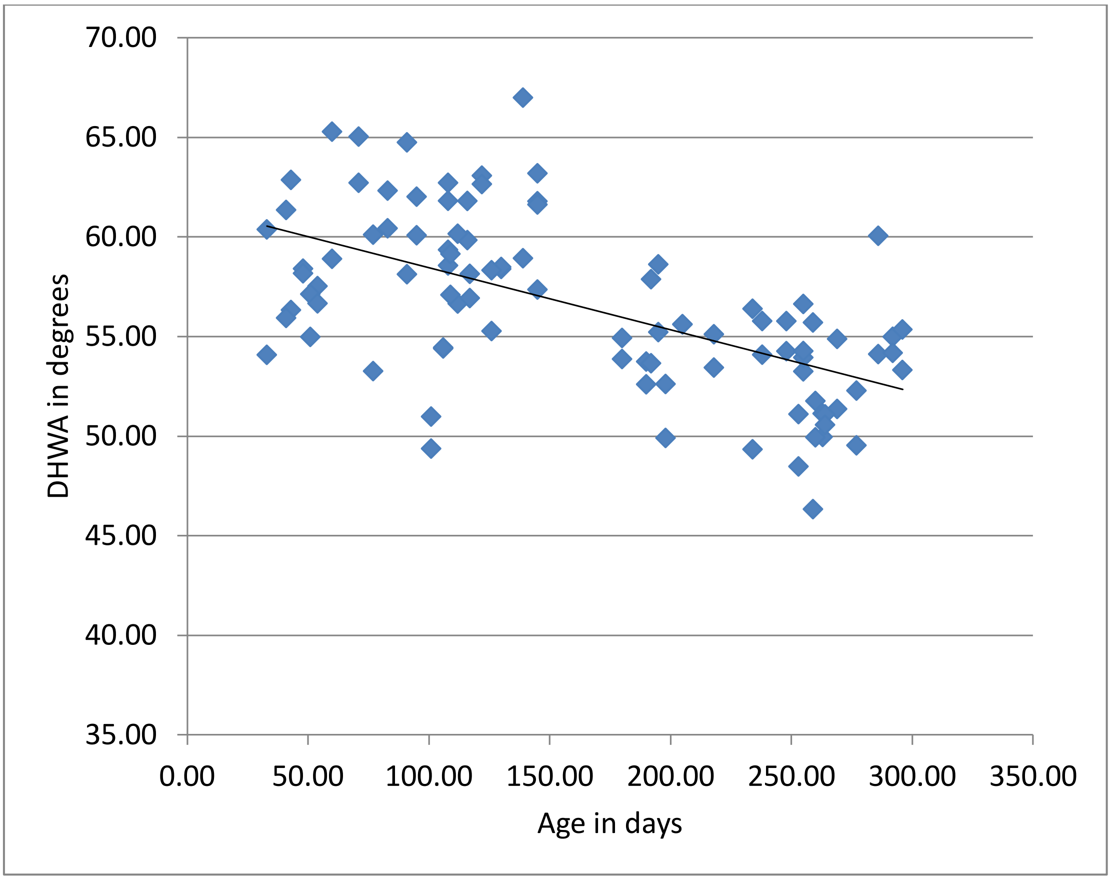


Figure 4.5: *The DHWA declined by a mean 5.9° between the two measurement dates (June and November); R^2 = 33.26%, P=0.001 (n=22).*

4.3.2: *Regression analysis*

There was a negative relationship between DHWA and age, *r* = -0.58, P=0.001. In both June and November the DHWA and DPA means differed significantly; June 6.1±2.1°, P=0.001; November 2.5±1.6°, P=0.001. The DPA was more acute than the DHWA but as the DHWA reduced with age this difference lessened. The integument depth increased both at the proximal and distal sites from June to November (Table 4.1). From June to November proximodistal differences in integument depth significantly lessened, P<0.001 so that the integument became more parallel. The relationship between age and proximodistal parallelism of the integument was also significant; *r* = 0.80, P=0.001.

Table 4.1: *June and November mean (±SD) morphometrics of the sole, dorsal hoof wall integument and distal phalanx of a TB foal population (n=22); significance, *<0.05, **<0.01, ***<0.001; LF = left front, RF = right front.*

	June Mean	June SD	November Mean	November SD	Absolute difference	% Difference
Age in days:	94	7.6	242	7.6	148	
Metrics in mm:						
Hoof Width left front	77.4	5.9	100.8	6.6	23.4***	130.3
Hoof Width right front	77.7	6.0	100.3	6.7	22.7***	129.2
Hoof Length left front	77.4	6.4	97.1	7.1	19.7***	125.4
Hoof Length right front	76.7	6.5	93.5	9.5	16.8***	122.0
Heel Width left front	49.9	6.0	60.8	5.1	11.0***	122.0
Heel Width right front	50.1	5.8	58.0	5.9	7.8***	115.6
Hoof Width by x-ray left front	72.2	5.3	92.9	4.4	20.6***	128.5
Hoof Width by x-ray right front	74.1	5.3	92.6	5.0	18.5***	125.0
Distal phalanx width left front	56.8	3.2	65.6	2.5	8.7***	115.4
Distal phalanx width right front	56.9	3.6	66.2	3.4	9.4***	116.4
Proximal Integument Depth left front	11.4	1.2	13.3	0.9	1.9***	116.8
Proximal Integument Depth right front	11.6	1.1	13.5	1.0	1.9***	116.0
Distal Integument Depth left front	8.2	0.8	11.8	1.3	3.6***	144.4
Distal Integument Depth right front	8.3	0.8	12.1	1.1	3.8***	145.9

Table 4.2: *June and November (±SD) radiological angles of phalanges and dorsal hoof wall of a TB foal population (n=22); significance; *<0.05, **<0.01, ***<0.001; LF = left front, RF = right front.*

	June Mean	June SD	November. Mean	November SD	**Absolute difference**	% Difference
Age in days:	94	7.6	242	7.6	148	
Angles by degree:						
Dorsal Hoof Wall LF	58.4	3.8	53.3	3.2	-5.1***	91.2
Dorsal Hoof Wall RF	59.7	3.5	53.7	2.4	-6.0***	89.9
Dorsal Parietal LF	52.8	3.3	50.6	3.3	-2.1***	95.9
Dorsal Parietal RF	53.1	3.1	51.3	2.3	-1.8***	96.7
Solar Parietal LF	22.0	2.3	18.6	3.2	-3.4***	84.8
Solar Parietal RF	21.8	2.6	19.4	2.6	-2.4***	88.8
Solar Border LF	6.5	2.0	4.4	1.8	-2.1***	67.3
Solar Border RF	6.7	1.7	4.7	1.7	-2.1***	69.3
Phalangeal axis LF	-13.5	8.8	-9.4	4.3	4.1**	69.5
Phalangeal axis RF	-11.1	7.1	-7.2	4.3	3.9**	64.9
Hoof pastern axis LF	5.6	2.3	2.6	1.7	-3.0***	46.9
Hoof pastern axis RF	6.6	1.8	2.3	1.4	-4.2***	35.5

4.3.3: *Hoof width differences (mm±SD)*

Individual foals were tested for asymmetry of hoof widths, LF to RF, in June and November. There was no significant difference in June or November between right and left feet (P=0.009). There were no significant correlations between hoof width differences and DHWA differences, despite absolute hoof width and DHWA showing a weak correlation in November (see Table 4.4).

Table 4.3: *Relationships between morphometric and radiological measurements recorded in June (n=22). Hoof width, HW; hoof length, Heel, heel width; HL; Hoof width radiograph, HWX; distal phalanx width, DPW; proximal integument depth, PID; distal integument depth, DID; dorsal hoof wall angle, DHWA; dorsal parietal angle, DPA; solar parietal angle, SPA; solar border angle, SBA;PA, phalangeal axis; hoof pastern axis, HPA** Correlation is significant at the 0.01 level (2-tailed), * correlation is significant at the 0.05 level (2-tailed).*

	Age	HW	HL	Heel	HWX	DPW	PID	DID	DHWA	DPA	SPA	SBA	PA	HPA
Age	1													
HW	.810**	1												
HL	.845**	.899**	1											
Heel	.682**	.788**	.696**	1										
HWX	.725**	.915**	.841**	.840**	1									
DPW	.773**	.878**	.833**	.709**	.915**	1								
PID	.727**	.755**	.717**	.695**	.668**	.622**	1							
DID	.736**	.618**	.684**	.560**	.556**	.628**	.677**	1						
DHWA	.187	.177	.115	.299*	.068	.072	.350*	.352*	1					
DPA	.030	-.045	-.025	.073	-.138	-.105	-.016	.238	.825**	1				
SPA	-.143	-.074	.012	.070	-.064	-.118	-.107	.154	.618**	.710**	1			
SBA	-.083	-.061	.004	.053	-.086	-.048	-.111	.146	.545**	.762**	.674**	1		
PA	.045	-.163	-.073	.070	-.190	-.163	.048	.298*	.582**	.651**	.551**	.531**	1	
HPA	.283	.382*	.241	.415**	.334*	.287	.642**	.255	.493**	-.085	-.004	-.212	.024	1

Table 4.4: *Relationships between morphometric and radiological measurements recorded in November (n=22). Hoof width, HW; hoof length, Heel, heel width; HL; Hoof width radiograph, HWX; distal phalanx width, DPW; proximal integument depth, PID; distal integument depth, DID; dorsal hoof wall angle, DHWA; dorsal parietal angle, DPA; solar parietal angle, SPA; solar border angle, SBA;PA, phalangeal axis; hoof pastern axis, HPA. ** Correlation is significant at the 0.01 level (2-tailed), * correlation is significant at the 0.05 level (2-tailed).*

	Age	HW	HL	Heel	HWX	DPW	PID	DID	DHWA	DPA	SPA	SBA	PA	HPA
Age	1													
HW	-.079	1												
HL	.124	.728**	1											
Heel	-.137	.664**	.601**	1										
HWX	.726**	-.235	-.148	-.232	1									
DPW	.526**	-.131	-.014	-.101	.760**	1								
PID	.263	-.340*	-.250	-.192	.501**	.535**	1							
DID	.464**	-.176	-.082	-.223	.516**	.415**	.792**	1						
DHWA	-.176	.342*	.389**	.283	-.193	.048	.030	-.025	1					
DPA	-.057	.439**	.469**	.308*	-.158	-.054	-.021	.189	.847**	1				
SPA	-.101	.346*	.355*	.235	-.110	-.073	.038	.281	.604**	.809**	1			
SBA	-.276	.179	.181	.126	-.372*	-.251	-.111	.045	.431**	.602**	.679**	1		
PA	-.129	.218	.213	.165	-.046	.133	-.050	-.122	.331*	.356*	.325*	.194	1	
HPA	-.212	-.190	-.158	-.055	-.058	.183	.092	-.391**	.243	-.310*	-.395**	-.328*	-.058	1

4.4: Summary of findings:

This study documented the morphometrics of the hoof capsule and phalanges in TB foals that occur due to growth and development in the first year of life. As hypothesised; 1) the phalangeal axes and HPA did not align; 2) the hypothesis that uneven DHWAs and hoof width differences were associated was rejected, 3) as hypothesised DHWA declined with age. In mature healthy horses the HPA was expected to represent the phalangeal axis (Balch *et al,* 1997). In this study it was confirmed that the HPA and phalangeal axes were not aligned in foals. Convergence of the HPA and phalangeal axes had not occurred by November, but appeared to be moving towards it. The DHWA of Thoroughbred foals has not previously been recorded at this early paediatric age and the decline during maturation to weanling age was a new finding. A discussion of the results is found in Chapter 7.

Chapter 5: Hoof renewal and hoof growth

5.1: Introduction

This chapter investigates the time that hoof renewal takes in newborn foals and weanlings. It also measures hoof growth in older foals from weanling to yearling and and describes a previously undiscovered factor affecting hoof wall growth. Two studies are described here where novel techniques were employed to measure hoof renewal and hoof growth. Study 1 measured hoof renewal from birth and study 2 calculated hoof renewal in a group of weanlings, their hoof growth rate (HGR) and compression of horn.

5.1.1: *Ethical agreement:* The stud farms' owners in both studies understood and agreed to the study. Ethical approval was given by the University of Central Lancashire Animal Projects Committee (RE/12/06/SH).

5.2: Study 1: Hoof renewal in newborn foals.

While a number of authors have guessed at the hoof renewal time for mature horses only one appears to have used data (Hickman, 1977; Kainer, 1987; Josseck, 1991). Josseck (1991) calculated hoof renewal in mature horses as 11 months, measuring Lipizzaner horses. A number of authors have speculated that the hoof wall grows faster in young horses but only two have measured hoof growth rates in foals (Smallwood *et al*, 1986; Butler and Hintz, 1977). Neither of these studies investigated the time required from birth to replace the hoof capsule or wall.

5.2.1: *Hypothesis*

The hypothesis was that hoof renewal in new born foals would take less time than in mature horses and the objective was to measure the time taken for the fetal hoof of newborn foals to grow to the bearing border and be replaced by horn generated since birth.

5.3: Method

A few weeks after birth a growth ring is present in the hoof wall of all four feet parallel to the coronary band, marking the event of foaling (Figure 5.1). From personal observation it has the appearence of a fold in the horn and is described here as a 'foal hoof crease' (FHC).

Figure 5.1: *The front feet of a foal of approximately two months age showing the foal hoof crease marking the time of birth.*

Thoroughbred foals from four stud farms (n=150) were assessed prior to and after routine hoof trimming at three week intervals. Each foal was noted as to whether the fetal hoof, distal to the FHC, was visible prior to hoof trimming. All hooves were trimmed by the author. Following hoof trimming the presence or absence of the FHC was determined in both front hooves. Where the fetal hoof was no longer visible post trim, the foal joined the cohort and the age of the foal was recorded (Figure 5.2). There was a large drop out rate, due to the method, where many showed the presence of the FHC prior to trimming and it remained after trimming.

5.3.1: *Statistical analysis*

The data were tabulated in Excel[4], analysed in Minitab 16[5] and assessed for normality using an Anderson-Darling test. Only data from foals with no history of lameness, illness or stable confinement were analysed.

Figure 5.2: *The remnant of the fetal hoof and FHC can be seen pre-trim on the left hoof (*a*); post-trim this is absent (*b*).*

5.4: Results (days±SD)

45 foals fulfilled the study criteria. The mean age at which the fetal hoof was removed by trimming was 145±15 days, range 120-165 (Figure 5.3).

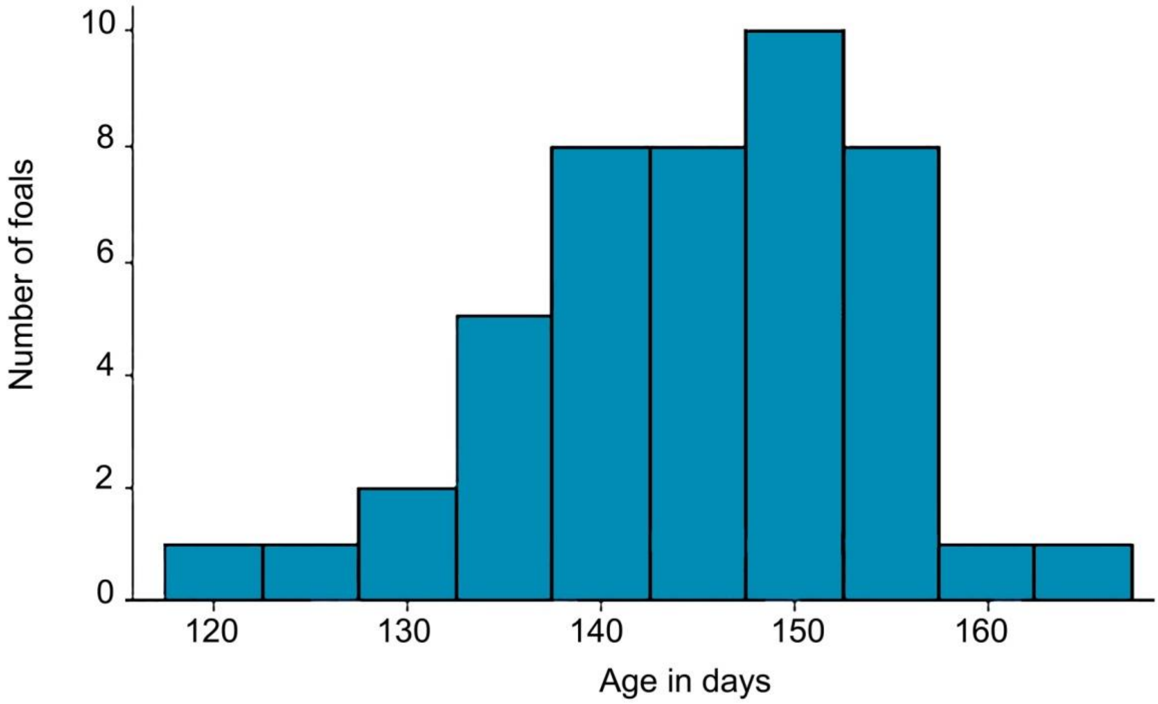


Figure 5.3: *Histogram of the age at which the foal hoof crease (FHC) was removed by trimming (n=45).*

5.4.1: *Qualitative observations*

The FHC was visible in all four hooves of all the foals viewed, although it varied in prominence. The colour of the horn occasionally differed between fetal and foal hoof where the foal, born with hooves of one colour, produced a different coloured horn *post partum* (Figure 5.4). Foals exhibiting signs of a flexural deformity affecting the distal interphalangeal joint often had an observable change in angle of the dorsal hoof wall at the FHC (Figure 5.5). The FHC was so named by the author due to its appearance which is dissimilar to other growth rings, seen at times on all four hooves. It appears more as a horizontal pleat in the wall rather than the usual prominent growth rings.

Figure 5.4: *A foal at approximately two months of age showing light coloured fetal hoof distal to the FHC and darker horn proximally.*

Figure 5.5: *Where foals have a flexural deformity, this may produce a change in dorsal hoof wall angle at the FHC.*

5.5: Study 2: The effect of plastic compression of horn upon hoof wall growth in weanling and yearling Thoroughbreds

The hoof growth rate (HGR) and hoof renewal of weanling/yearlings has not been measured in Thoroughbreds. The only study of horses of a similar age was of 14 Shetland ponies; however this study did not measure hoof renewal (Butler and Hintz, 1977). Currently, the factors influencing hoof capsule shape change are thought to be associated with; hoof growth, wearing at the bearing border, farriery, and plastic deformation but little scientific evidence exists to substantiate these theories (Figure 5.6). Hoof shape may be altered by differing hoof growth rates at various sites around the hoof capsule and given that hoof shape has been shown to alter compression forces in the hoof wall this may mean that HGR is influenced by loading (Thomason *et al*, 1998; Faramarzi *et al*, 2009).

Figure 5.6: The club foot (closest) of a foal of approximately three months with a number of characteristics at variance with the opposing front foot; broken forward (positive) HPA, higher heels, steeper dorsal wall.

5.5.1: *Hypotheses*

It was hypothesised that: 1) HGR would be slower in weanlings than in young foals; 2) hoof renewal would take longer than in newborn foals; 3) plastic compression of hoof horn would occur over time. The aims of the study were 1) to measure hoof growth and hoof length in Thoroughbred weanling/yearlings, 2) to record hoof renewal time at this age and 3) to determine whether the hoof wall compresses plastically over time.

5.6: Method and Materials:

5.6.1: *Selection of horses*

Twelve Thoroughbred (TB) weanlings were selected with the following criteria; they were born in the same year at one of two stud farms and were healthy individuals with no history of lameness. They were fed well balanced diets, turned out on grass pasture during the day and stabled at night. All weanlings were controlled by experienced handlers and monitored throughout the measurement procedures to ensure that no undue stress was caused. Ethical approval was given by the University of Central Lancashire Animal Projects Committee (RE/12/06/SH).

5.6.2: *Morphometric procedure*

The study began for all weanlings within three days, using the left front to take measurements. The methods developed by the author, recorded in 2.6.1, Chapter 2 were further improved for this study. The author trimmed the feet immediately prior to each measurement. The solar plane of the hoof wall was trimmed to approximately 3mm from the cleaned sole at 90° to the long axis of the cannon, aiming for an aligned HPA. The distal border was rounded to an approximate 3mm radius. No rasping of the outer wall took place. A sagittal line was drawn through the frog to the toe and thereon up the dorsal wall; this gave the mid-line sagittal centre (Reilly *et al*, 1998[b]). On this line a 2mm diameter hole was drilled to 2mm depth approximately 15mm distal to the coronary hairline (CH) and another approximately 15mm distal to the first. The holes were filled with an acrylic[8] of different colour to the hoof and allowed to set. The hardened acrylic was filed flush with the hoof wall creating two clearly defined circular marks (Figure 5.7). A disc marker of 12.7mm was attached adjacent to the two acrylic marks. The CH was marked with a felt-tipped pen in red to define it. With the hoof lifted and extended, so that it was non-weight bearing, 5 photographs were recorded with the camera lens in the same plane as the dorsal wall at 90° to the marker from a distance of approximately 30cm, on digital camera[7]. The photographs were checked, on camera, for framing and focus and the best selected, if necessary more were taken. The most suitable image was downloaded to a recognised software[9] designed to measure hooves (White *et al*, 2008). This was repeated at approximately four week intervals until the proximal mark was removed by wear or trimming at the bearing border.

5.6.3: *Photometry*

Each image was calibrated in millimetres using the circular marker and linear dimensions were recorded three times with the annotations invisible. The variables measured were; distance between the CH and the centre of the proximal acrylic mark, between the centres of both acrylic marks, and CH to bearing border (Figure 5.8). Distances were then revealed and tabulated in Excel[5].

Figure 5.7: The two 2mm radius holes were filled with hoof repair acrylic[8] *to produce circular marks which remained until they reached the bearing border of the hoof wall.*

5.6.4: *Calculations*

The distance from the CH to the proximal acrylic mark at each data collection was used to measure hoof growth and to calculate hoof growth rate (HGR), hoof growth was divided by the number of days measured. Hoof length was measured from the CH to the bearing border. The distance between the acrylic marks, at the first data collection, was given a value of 100% and following measurements were calculated as a percentage change in distance between the same two marks. This was recorded as the compression percentage (*C%*).

Hoof renewal time was determined in days as the sum of the recorded hoof growth time plus the calculated time of the proximal and distal unmeasured hoof remnant (*Ur*) time. The unmeasured hoof remnant was multiplied by the compression percentage (*C%*) divided by the HGR for that individual.

$$Ur \times \left(\frac{Cr\%}{100}\right) = Compressed\ Ur$$

$$\frac{Compressed\ Ur}{HGR} = Ur\ growth\ time$$

$$recorded\ hoof\ growth\ time + Ur\ growth\ time\ = hoof\ renewal$$

Ur = unmeasured remnant; *Cr%* = compression residual percentage, *HGR* = hoof growth rate.

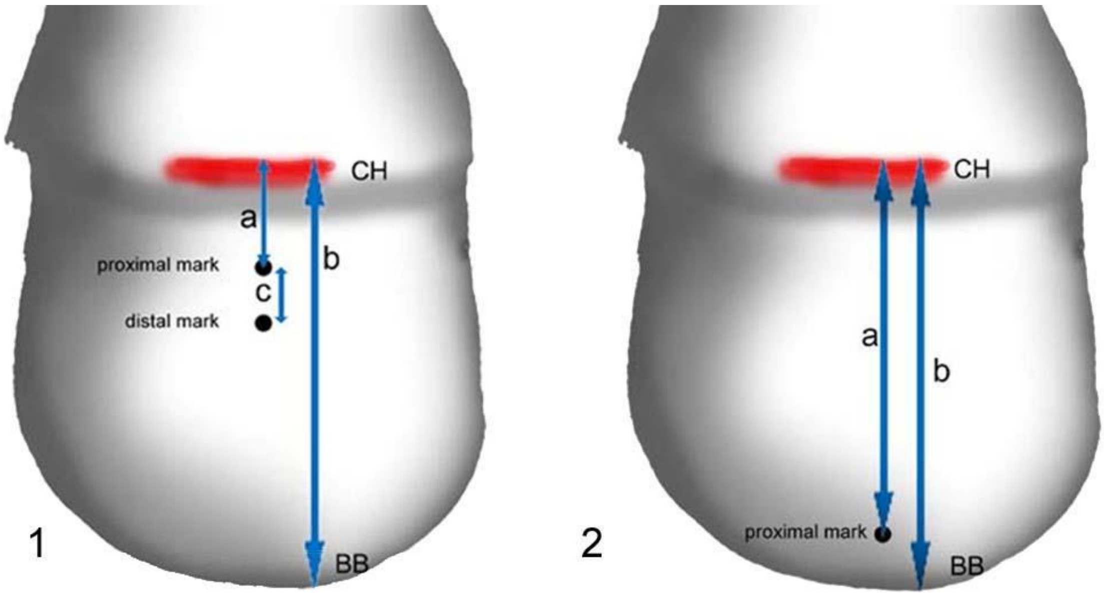


Figure 5.8*: Three variables were initially measured; 1, a) the distance from the coronary hairline (CH) to the centre of the proximal mark, b) the distance from CH to bearing border (BB), c) the distance from the centre of the proximal mark to the centre of the distal mark; 2, eventually the distal mark disappeared and only variables* a *and* b *continued to be measured.*

5.6.5: *Statistical analysis*

Descriptive statistics were used to assess data distribution and variation using Minitab 16[5] and tested for normal distribution using an Anderson–Darling test. Regression analysis was used to test hoof growth and compression with significance set at P<0.05. A paired *t*-test was used to compare original hoof length to final hoof length.

5.7: Results

The mean±SD age (in days) of the weanlings when first measured was 285±26, range 253-324. The mean age of the yearlings when last measured was

413±31, range 358-478. The mean number of days that each horse was measured was 155±23, range 112-184. A two sample *t* test was used to test for difference in means between the weanling/yearlings from the two different studs. There was no statistical difference for HGR, P=0.883; hoof renewal, P=0.344, hoof compression, P=0.844; nor toe length, P=0.426.

5.7.1: *Hoof growth rate (mm/day±SD)*

The mean hoof growth rate for the population was 0.25±0.06mm/. Hoof growth was positively correlated to age (*r* = 0.88, P=0.001).

5.7.2: *Hoof length (mm±SD)*

Mean hoof length (mm), increased during the measurement period from 69.23±4.8mm to 77.5±4.3mm. Final hoof lengths were found to be significantly longer than original hoof lengths (*t* = 8.3, [6.1, 10.5] P=0.001), the correlation of hoof length to age was *r* = 0.63, P=0.001.

5.7.3: *Hoof renewal (days±SD)*

The hoof renewal times were normally distributed (AD = 0.26, P = 0.63). Hoof renewal in days was 283±29, range 259-319.

5.7.4: *Hoof compression (mm/day± SD)*

The mean compression was 0.03±0.016mm per day. There was a relationship between an increasing amount of plastic compression of the hooves to the time point that they were measured; *r* = 0.46, P = 0.002.

5.8: Summary of findings

5.8.1: *Study 1*

Thoroughbred foals in the first study in this chapter renewed their fetal hoof wall at twice the rate calculated in mature horses (Josseck 1991). The hypothesis that newborn foals renew their hooves faster than mature horses was therefore confirmed. This was not a surprising finding as the foot of a foal is smaller and therefore there is shorter distance for the hoof to grow from the site of origin at the coronary band to the bearing border. Additionally it has been reported that the foal hoof grows faster than mature horses (Butler and Hintz, 1977; Smallwood *et al*, 1989).

5.8.2: *Study 2*

The second study in this chapter investigated hoof growth in weanling/yearling Thoroughbreds to determine the time of hoof renewal and also whether hoof growth was influenced by plastic compression due to weight-bearing. In accordance with the hypotheses, hoof growth rate was slower compared to that of young foals; 0.25±0.06mm/day compared to 0.42mm/day (Smallwood *et al*, 1989) and hoof renewal took longer; 284±29 days compared to newborn foals, 145±15 days (Curtis *et al,* 2014). The third hypothesis, that plastic compression of the hoof wall would occur over time, during hoof wall descent, was confirmed.

Chapter 6: The effect of regional solar loading upon hoof growth

6.1: Introduction

This chapter investigates differential hoof growth around the wall and considers associations with hoof compression and solar loading. Foals appear to be born with symmetrical and even front hooves which by maturity are individually asymmetric and often have uneven dorsal hoof wall angles and this is considered an undesirable trait (Ducro *et al*, 2009). The processes and causes of these changes are poorly understood (Bidwell and Bowker, 2006). Non-weight-bearing at the heels in foals is a recognised diagnostic sign of a flexural deformity affecting the distal interphalangeal joint (AFDdipj) and seen as a pre-curser to a club foot (Owen, 1975; Barr and Kidd, 2002, Curtis *et al,* 2012).

6.1.1: *Study aims*

The aims of this study were to quantify and compare; hoof growth and hoof compression in the hoof wall, solar hoof loading, and dorsal hoof wall angle (DHWA), in a control group of healthy foals and a group of foals with AFDdipj. The objectives were to; 1) record the hoof growth rate (HGR) and typical DHWA of young foals; 2) measure hoof growth and compression of horn in the medial, dorsal and lateral hoof walls of both front feet; 3) quantify load distribution at the solar hoof surface; 4) compare HGR, hoof compression, DHWA, and loading in both groups.

6.1.2: *Hypotheses*

The hypotheses were, 1) that hoof growth differs around the hoof wall, 2) that compression of horn affects hoof growth measurements, 3) foals with an AFDdipj differ in loading and hoof growth from healthy foals, 4) DHWA would be less in the healthy group compared to the AFD group, 5) that foals with AFDdipj can be identified visually and differentiated from healthy foals.

6.2: Methods:

6.2.1: *Selection of horses*

A convenience group of 18 Thoroughbred foals was selected from two stud farms to be the control group. Selection was based on the following criteria; the first available that were healthy, sound, and had no behavioural difficulties or major conformational faults. Major conformational faults were defined as those which had; veterinary diagnosis of congenital flexural deformities, varal and valgal deformities which required treatment, deformities which required farriery treatment beyond routine trimming. The treatment group (n=9) were selected from foals identified as having symptoms of AFDdipj by the author, age range 45-81 days. All foals in the treatment group were identified visually by lack of front feet heel weight-bearing, when viewed on a hard level surface, as described by Barr and Kidd (2002). All foals were controlled by experienced handlers and monitored throughout the measurement procedures to ensure that no undue stress was caused. The stud farms' managements understood and agreed to the study. Ethical approval was given by the University of Central Lancashire Animal Projects Committee (RE/12/06/SH).

6.2.2: *Morphometric procedure*

All morphometric procedures were carried out by the author. Each foal was marked on both front hooves using an adapted bit developed in a pilot study (Chapter 2) and also used in Chapter 5 (Figure 6.1). A hole of 2mm depth and diameter was drilled approximately 15mm below the coronary hairline and another in the same tubular alignment approximately 10mm below the first. The holes were filled with an acrylic hoof repair glue[8] of a different colour to the hoof. The markings were at three sites around the hoof wall; medially at the quarter, dorsally at the dead centre, laterally at the quarter and a calibration disc of known dimension (12.7mm) was attached adjacent to the marks.

Figure 6.1: *A 2mm diameter drill bit was reduced in length and set so that only 2mm protruded from the chuck.*

6.2.3: *Photometry*

The hoof was photographed in the manner developed in Chapter 2 and described in 5.6.2. A standing lateral view of each front foot was also recorded. Photographs were downloaded to recognised software[9] and measured three times on the blind setting after calibrating distances from the attached disc. The mean of the three measurements was recorded in Excel[5]. Hoof growth was measured from the coronary hairline to the centre of the proximal mark and subsequent measurements used to calculate hoof growth rate (HGR). Compression of horn was measured from the centre of each mark; the first recording was given a value of 100% and subsequent measurements calculated as a percentage and this was then divided by days measured to give the compression rate (HCR).

6.2.4: *Pressure mat*

The foals were walked onto a pressure mat[10] where their front limb loading was recorded at 50 hertz (Hz) for 20 seconds. No attempt was made to move or alter the stance of the foal. Three separate recordings were taken. Each recording was downloaded to a software programme for analysis[10]. The recording with the least sway was independently selected and frame 500 was used for analysis.

The dorsopalmar axis of each hoof was ascertained using image editing software[2] (Figure 6.2). Each selected frame was downloaded into Excel[5] and the left and right solar loading values isolated. These were then downloaded to the image software[2] and xy axes were situated in the geometric centre of the pattern; the axes were rotated to the predetermined dorsopalmar axis (Figure 6.3). The force (N) values of each quadrant were recorded. The quadrants were summed as lateral, dorsal, medial and caudal solar hoof regions and recorded as an intra-foot percentage (Figure 6.4).

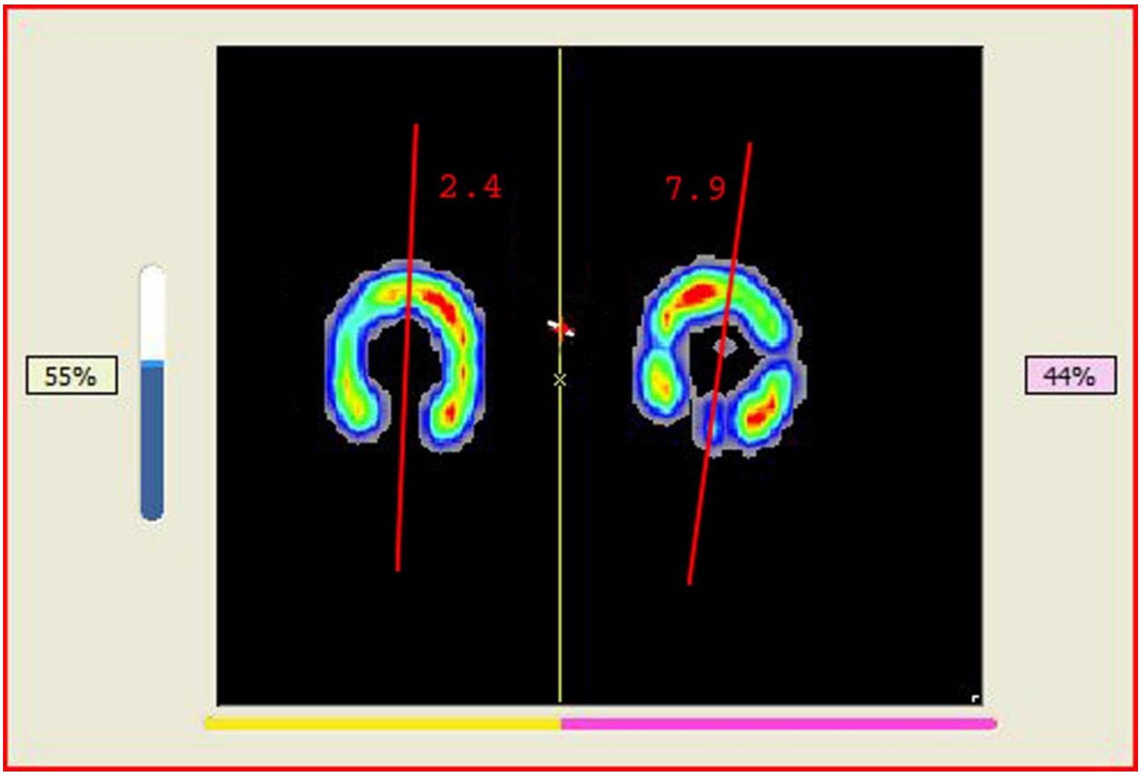


Figure 6.2: *Image capture from the pressure mat video allowed the dorsopalmar axis of both feet to be established; values in red gave the axial degrees of rotation; the red mark between the feet was the centre of pressure and the short white line by it showed the amount of sway during the 20 second data collection.*

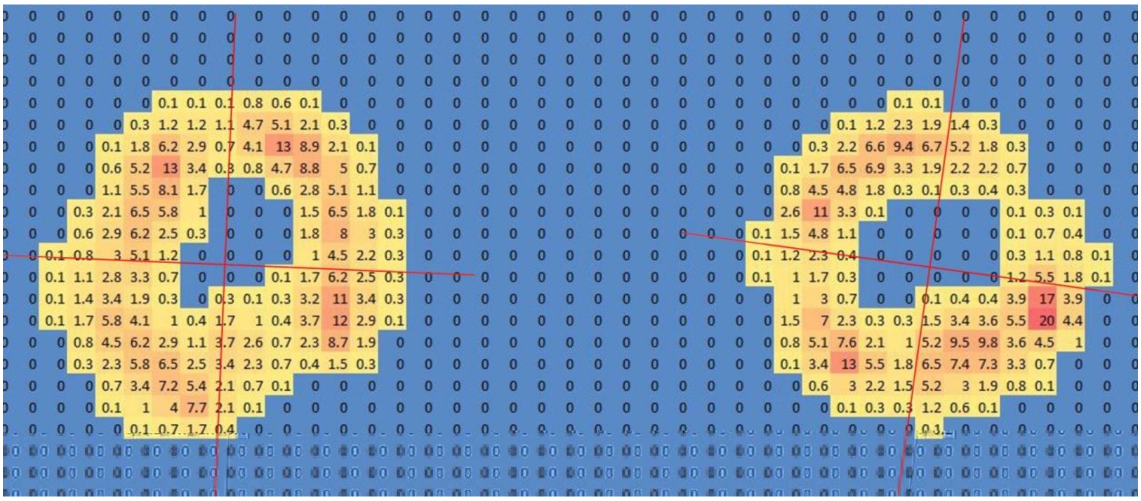


Figure 6.3: *The spreadsheet data was coloured to accord with values, a cross (y and x axes) was positioned in the geometric centre of data and rotated by the previously establish degrees.*

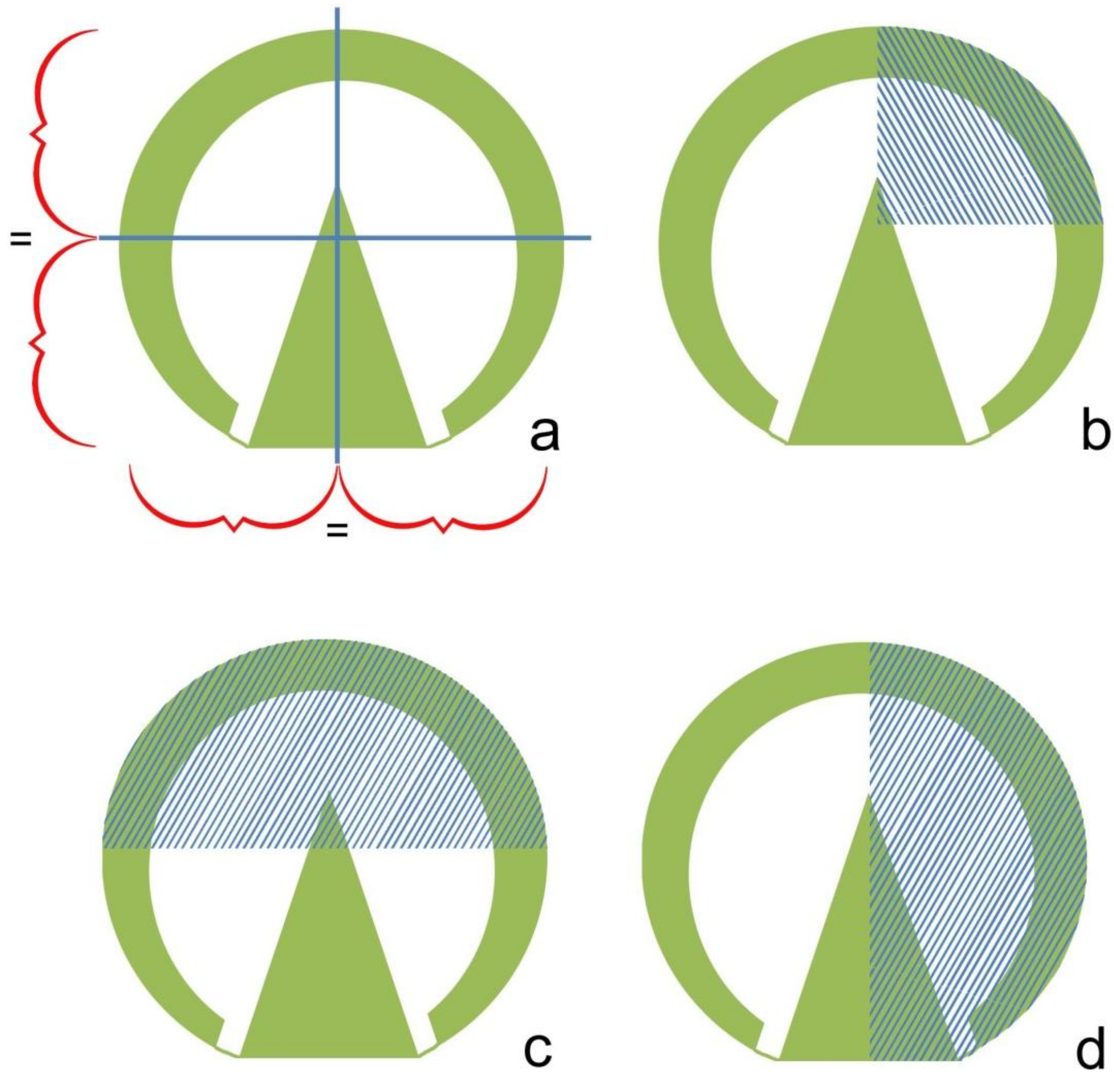


Figure 6.4: *a) The sum of the N values for each quadrant was recorded, b) the quadrant value was calculated as a percentage of the sum of all four quadrants, c) quadrant percentages were summed to produce caudal and dorsal solar regions and d) medial and lateral solar regions.*

6.2.5: *Statistical analysis*

Data were recorded in four categories; solar hoof loading, hoof compression, hoof growth, and DHWA. All data were tested in Minitab 16[6] for normal distribution using Anderson-Darling with significance at $P>0.05$. Initial exploratory analysis of all the variables took place using Principal Component Analysis (PCA) and Pearson correlation. Paired *t*-tests were used to compare intra-group left front with right for foot and independent sample *t*-tests for control group with the AFD group. The HCR, HGR, and solar hoof loading data were individually tested using ANOVA.

6.3: Results

6.3.1: *Principal Component Analysis (PCA)*

The variables, hoof growth and % loading were spread along the PC1 x axis and explained 29.0% of the variation. Separation along the y axis of PC2 of compression and DHWA explained 26.3% of the variation Figure 6.5). The control group and AFD group showed separation; the control group of foals toward hoof compression and the AFD group toward DHWA. From these results further correlation analysis between the variables was carried out and is reported later.

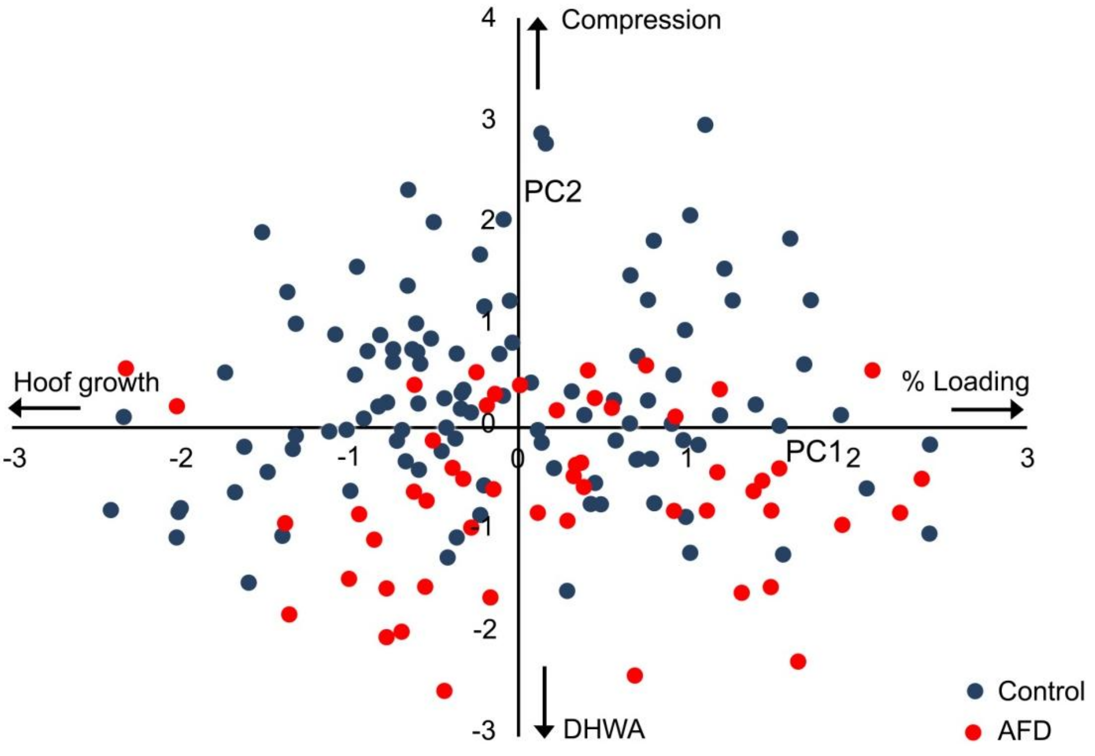


Figure 6.5: *Principal component analysis; along axis PC1, % Loading and Hoof growth show the greatest positive (% Loading) and negative (Hoof growth) effect on each other. When graph points were coloured according to categories; control blue and AFD red, the separation occurred along PC2. The control (n=18) data points were clustered around the compression end of the axis whilst the AFD (n=9) individuals were clustered at the DHWA end.*

6.3.2: *Hoof growth rate (mm±SD)*

The mean HGR per day for all regions was; for the control group, 0.41mm±0.1mm, P=0.73 and the AFD group 0.42mm±0.08mm, P=0.79. A 2-sample *t*-test comparing the two groups showed no significant difference in mean HGR, P=0.575. There were no difference of means between the LF and RF in the control group, P=0.379. The AFD group showed a difference between LF dorsal and RF lateral, P=0.004, (Figure 6.6). Inter-group differences between LF control and LF AFD were significant, P=0.030; and between RF control and RF AFD, P=0.019.

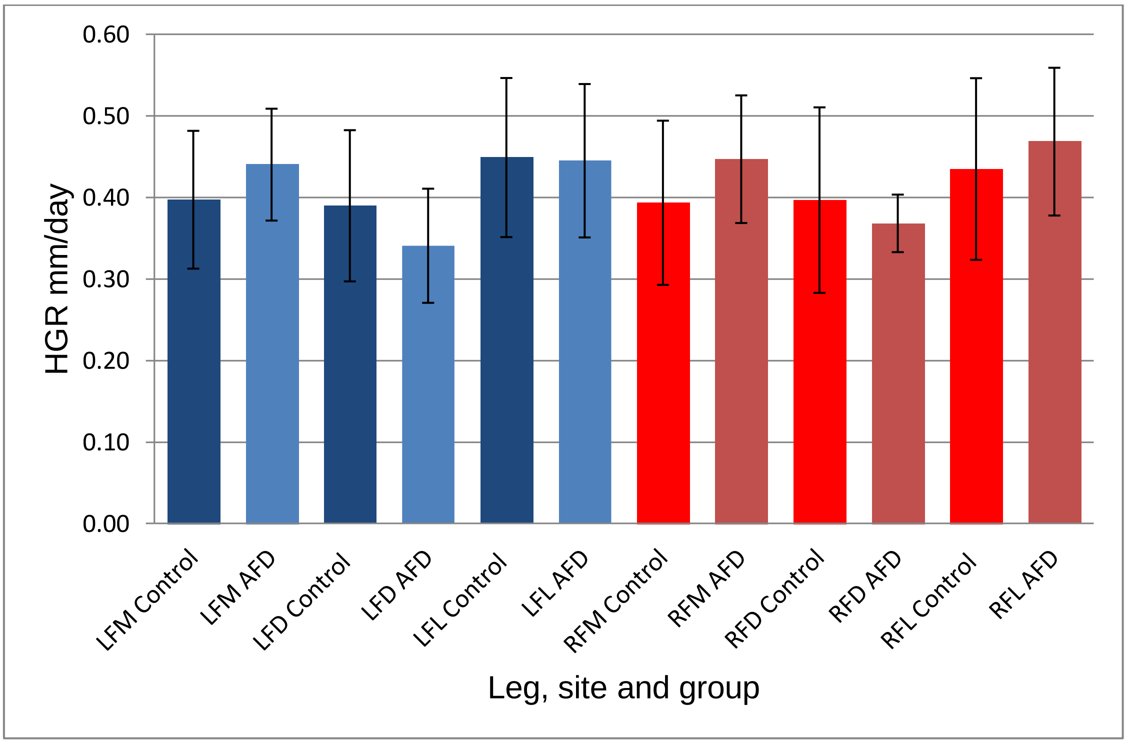


Figure 6.6: *Control LF in dark blue, control RF in red, AFD LF in light blue, AFD RF in pink; M=medial, D=dorsal, L=lateral; bars giving standard deviation. Medial and dorsal HGR were significantly less than lateral HGR in both control front hooves; dorsal HGR was significantly less than medial and lateral HGR in both AFD front hooves.*

6.3.3: *Dorsal hoof wall angle (°±SD)*

The mean DHWA for the control group was 58.2±2.9°, P=0.735; the AFD group was 60.1±3.3°, P=0.791. The DHWA in the control group was significantly less than the AFD group, -1.9, P=0.006. The intra-group differences between left and right were not significant; control group P=0.171, AFD group P=0.277.

6.3.4: *Compression of horn (mm/day±SD)*

The mean HCR in mm per day, for the control group was 0.039±0.022mm/day, P<0.005; the AFD group was 0.028±0.013mm/day, P<0.005. Comparing the two groups showed that HCR in the AFD group was significantly less than the control group, 0.012mm/day, P<0.05. The control group showed a difference between LF lateral and LF medial, P=0.031, in the AFD group the LF lateral and RF dorsal differed, P=0.011 (Figure 6.7). The LF and RF of the AFD group showed significant differences; LF P=0.015, RF P=0.008. Individual intra-foot HCR showed differences in the control LF, P=0.012, the AFD LF, P=0.016, and AFD RF, P=0.022. The control RF showed no difference P=0.387.

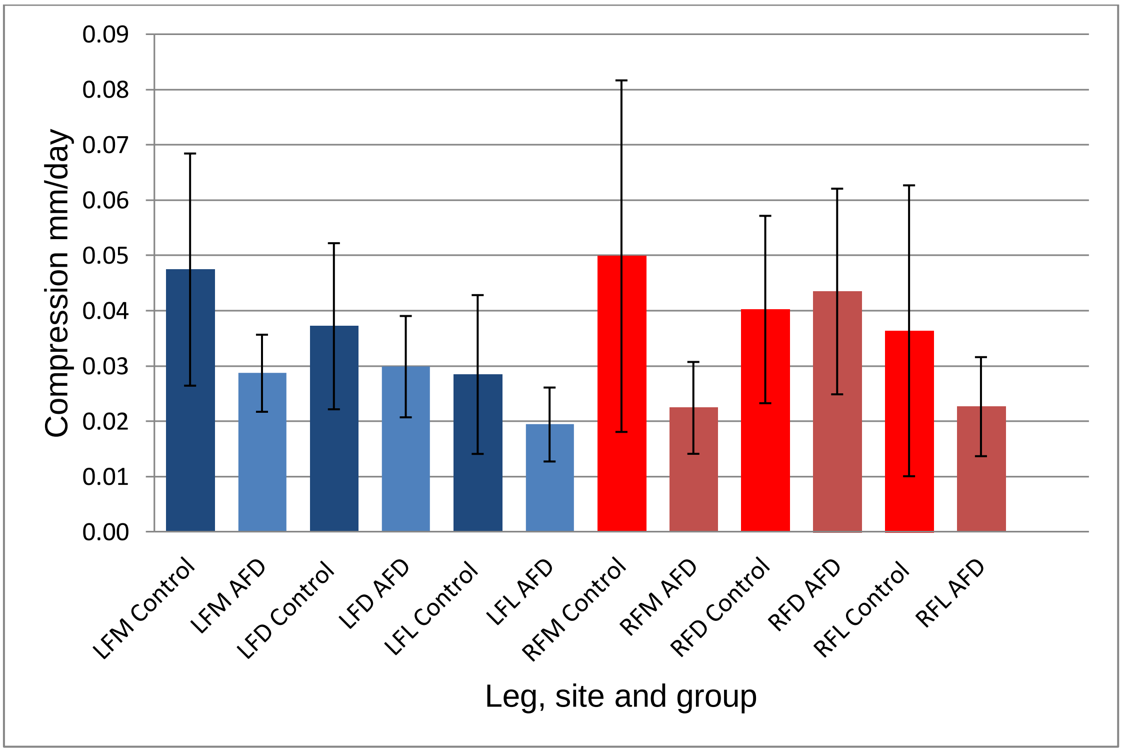


Figure 6.7: *Control LF in dark blue, control RF in red, AFD LF in light blue, AFD RF in pink; M=medial, D=dorsal, L=lateral; bars giving standard deviation. The pattern of hoof compression was similar in both LF and RF in the control group; in the AFD group hoof compression was greatest in the dorsal hoof wall; it was significantly greater in the RF.*

6.3.5: *Solar hoof loading (%±SD)*

The mean percentage loading for the control group was 50±14.6%, P =0.006; AFD group, 50±18.6%, P=0.69. The solar hoof regions showed significant differences in loading in both the control and AFD groups, P=0.001; intra-foot differences were also significant, P=0.001 (Figure 6.8).

6.3.6: *Correlation analysis*

There was a positive correlation of dorsal loading to DHWA, r=0.30, P=0.026; and a negative correlation of hoof growth to loading, r=-0.18, P=0.019.

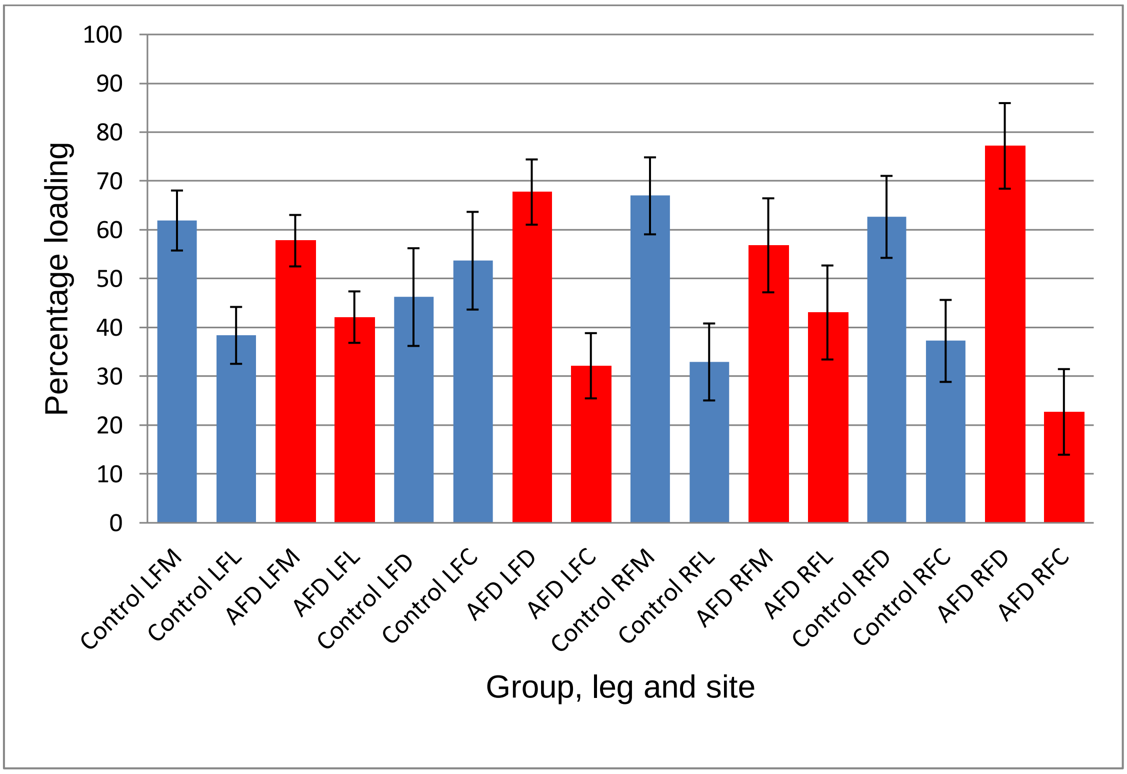


Figure 6.8: *Control in blue, AFD in red; M=medial, D=dorsal, L=lateral, C=caudal, bars giving standard deviation. Distribution of loading, by percentage of the solar region in the control group showed significant differences medial to lateral LF and RF and dorsal to caudal RF but not LF dorsal to caudal. The AFD group showed significant differences medial to lateral LF and dorsal to caudal LF and RF; RF medial to lateral did not significantly differ.*

6.4: Summary of findings

This study investigated hoof growth, dorsal hoof wall angle, hoof compression and solar hoof loading in young foals. In accordance with the hypotheses, hoof growth rate differed around the hoof wall and this was related to loading; foals with a flexural deformity differed in loading and hoof growth rate from the control group of healthy foals. The hypotheses that the DHWA would be less in the healthy group compared to the AFD group and that foals with AFDdipj could be identified visually were confirmed.

The mean overall hoof growth rate did not differ between groups but there were significant differences at the three sites and this may be a partial explanation for the asymmetrical solar shape of mature hooves and also the difference between medial and lateral hoof wall angles (Roland *et al,* 2003; Faramarzi *et al*, 2009). The foals with an acquired flexural deformity showed a pattern of hoof growth in both limbs where dorsal HGR was significantly less than medial and lateral HGR and this may partially explain how the evenly matched hooves found in neonate foals become uneven in maturity. These are original findings which could influence treatment strategies aimed at constraining and/or reversing hoof distortion.

Chapter 7: Discussion

This chapter reviews the findings of Chapters 3 to 6 and discusses the implications of their results.

7.1: Chapter 3, structure of the epidermis in fetuses and foals

This study investigated the epidermal hoof wall in fetuses and paediatric foals. There appeared to be three possible factors to explain the process of epidermis widening; 1) an increase in horn tubule numbers, 2) an increase in horn tubule diameter, 3) a reduction in tubular density, or a combination of these. The results confirmed that with aging the epidermis thickened and horn tubules increased in diameter, also as the epidermis widened the density of the tubules decreased.

As the time of hoof renewal of a newborn foal is 145 days (Chapter 5), most and probably all samples of hoof used in this study, were developed *in utero*. Although the dorsal hoof wall was thicker than at the quarters, there was not a difference in thickness between medial and lateral found in mature horses. The change from symmetry to asymmetry may be an evolutionary adaption or a response to the uneven loading of the hoof wall *post partum*. If the epidermis is destined to become thicker medially then horses that are fetlock varus and/or toe-in would be poorly prepared to potentially load more on the lateral wall. If, however, the epidermis responds to the stimulus of loading then horses with toe-in conformation would develop a greater thickness laterally and this may occur throughout life. The loading and hoof growth of the same three regions; medial, dorsal, and lateral, were investigated in the study described in Chapter 6 and will be discussed later in this chapter.

The very small size of the oval Type 3 tubules could not be accurately measured and so it was not established in this study if the size of these changed with the maturing foal. The largest tubules were all Type 2 which were found in the region of the *stratum medium* beginning approximately 10% of the epidermis thickness from the lamellae. It is possible that new larger horn tubules are added to the epidermis or established tubules increase in size. Horn tubules could be added in any area but it appears more likely that thickening occurs by additional horn tubules developing in the axial hoof wall adjacent to the lamellae. If this is the case then the Type 1 tubules may be immature Type

2 tubules, implying that new horn tubules contributing to epidermis widening are generated on the axial border of the *stratum medium*.

7.1.1: Horn tubule density

Horn tubule density did not differ significantly between medial and lateral, whereas Lancaster (2013) found that in mature horses the medial quarter had a greater density. This may again be a sign that the hoof wall is not pre-adapted but changes *post partum*, once the foal is loading the hoof. In this study the tubule density decreased with age while the specific number of tubules increased. As the size of tubules increased in diameter and density decreased with age then the density of intertubular horn may have remained the same or increased. Intertubular horn has a greater resistance to crack formation and an increase in its density may be related to this function (Bertram and Gosline, 1986). Horn tubule density was less at the dorsum than the quarters suggesting greater intertubular horn in this area. Anecdotal data suggests that cracks in paediatric foals occur at the dorsum, and this implies that this is the area most exposed to fracturing. In mature horses the tubular structure differs from foals, where the least tubular density is found at the lateral quarter, and therefore the intertubular horn density should be greater at this point (Lancaster, 2013). It is possible that this means that the hoof wall is adapted to change, with maturation, to the need to weight-bear more medially but resist cracking laterally.

7.1.2: Tubule type

It proved problematic to identify and therefore accurately classify all tubules into the three types, since although it was always possible to select an individual typical horn tubule; large numbers had the characteristics of two types (Figures 1.4 and 2.3). Adjacent to the lamellae some small tubules could be readily identified as Type 1 but there was not a defined distance from the lamellae nor diameter where they could they be readily classified as Type 2. Further into the centre of the *stratum medium*, the tubules gradually reduced in size and became more oval but again there was no defining point where Type 2 became Type 3. These Type 2/Type 3 tubules lay in the middle region which Leach (1980) designated the intermediate zone and is identified in this thesis as Type 4 (Figure 2.3). Reilly *et al* (1998[a]) ignored tubule types and showed four regions of differing density which did not coincide with the three tubule types (Leach, 1980). The studies contained within this thesis focus primarily upon hoof wall growth and development rather than mechanical properties and it was for these reasons that in this thesis horn tubules were not differentiated and all were counted during data collection and analysis. This allowed comparisons with previous studies of different breeds and ages and with similar regions of the hoof wall (Leach, 1980; Reilly *et al*, 1998[a]; Lancaster *et al,* 2013). The overlapping tubule structure of changing shape, diameter and density illustrates the heterogeneous nature of the hoof wall and is an example of its design which allows it to fulfil its many functions of weight-bearing, flexibility, crack resistance and durability.

7.1.3: Budding lamellae

The impression of tubules budding from lamellae tips, described by Lancaster *et al* (2013), was seen in many of this study's slides (37%) but not all (Figures 1.7 and 3.7). However, as the horn tubules are formed from the papillae at the germinative layer at right angles to the lamellae, it does not seem possible for lamellae to produce new tubules mid-wall. There may be a mechanism or stimulus, in the *stratum germinavatum* in the area where the lamellae link the *stratum internum* with the *stratum medium*, which encourages new papillae to develop adjacent to a lamella tip. While both papillae and lamellae are innervated, the proprioceptive mechanism involved, if any, appears to be unknown. The apparent flow of tubules away from the lamellae toward the centre of the *stratum medium* may be visual evidence of the epidermis widening axioabaxially.

7.1.4: Conclusions drawn from this study (Chapter 3)

The conclusions drawn from this microscopy study were that epidermal hoof wall thickness increases during development by no single means. The horn tubules increase in number and diameter while their density decreases and therefore the intertubular horn must increase. Mediolateral hoof wall thicknesses, tubule diameter, and tubule density, are all symmetrical in the mediolateral plane at this age; which is an original finding. Further research into the tubular structure of the hoof could compare epidermis, selected from the cadavers of mature horses with known conformational faults, to the results in this thesis. This may show whether the epidermal structure is pre-adapted to change during maturation or responds to different loading.

If the epidermal hoof wall can be shown to alter in response to loading then this opens new opportunities for corrective farriery for mature horses. Hoof distortion has been linked to unsoundness and therefore the ability to manipulate the hoof capsule to a more appropriate shape for an individual may promote improved soundness. Farriers have the ability to alter loading by trimming alone, and can increase this effect by the additional use of shoes that amplify biomechanics such as Bar Shoes and Lateral Extension Shoes. Future studies should investigate the effects of trimming, shoe type and application.

7.2: Chapter 4, Radiographic and morphometric study

This chapter described a study which documented morphometrics of the hoof capsule and phalanges in Thoroughbred foals that occur during growth and development in the first year of life.

7.2.1: *Hoof pastern axis and phalangeal axis*

In mature healthy horses the hoof pastern axis (HPA) was expected to represent the phalangeal axis (Balch *et al*, 1997). This study found that the HPA and phalangeal axes were not aligned in foals. Convergence of the HPA and phalangeal axes had not occurred by November, when the foals were between six and nine months old, but appeared to be moving towards it. Many foals are born slightly flaccid, bearing weight disproportionately on the caudal region of the hoof capsule, and then strengthen through exercise becoming similar to mature horses (Adams and Mayhew, 1984). There is considerable variety in the age that this occurs so that at one month visual assessment shows some foals still have a toe-up conformation and others, of the same age have toes with ground contact and a steep aligned dorsopalmar conformation (Curtis and Stoneham, 1999) (Figure 1.11). The data measuring phalangeal alignment recorded the incidence of this phenomenon. Using the HPA as a means of assessing horse conformation can be traced back to written advice over two thousand years ago, and remains current (Xenophon, 2008; Weller *et al,* 2006). The visual assessment of foals is unlikely to be replaced by the widespread use of radiography in the near future so this difference between external and internal alignment should always be considered.

There would appear to be no advantage to a foal having a negative phalangeal axis during its paediatric phase. From the author's experience, the signs range from sub-clinical to detrimental and injurious to the foal. It is not known whether feral foals experience the same problems and what the outcome is in severe cases. Thoroughbred farms manage foal exercise individually and farriery intervention is only required in severe cases (Curtis and Stoneham, 1999). Hoof shape is affected by severe cases of this condition, where the solar outline widens caudal to the quarters; this is temporary and the shape returns to normal some weeks after resolution of the condition (Figure 7.1).

Figure 7.1: *When a foal has suffered from hyperextension of the fetlock and phalanges the solar outline is distorted outwards caudal to the quarters.*

7.2.2: *Dorsal hoof wall angle*

Anderson and McIlwraith (2004) showed that Thoroughbred weanlings (approximately six months old), had a mean dorsal hoof wall angle (DHWA) of 55.3° descending to 51.9° as yearlings, whereas this thesis showed foals with a mean age of 94 days had a DHWA of 59.4±3.8° which descended to 53.3±3.2° at a mean age of 242 days. Although Anderson and McIlwraith (2004) were less than specific in recording the ages of their horses and a different methodology was used, there appears to be a consistency in the decline of the DHWA through both studies (Figure 7.2). Previous authors have recorded DHWA reductions in non-Thoroughbred foals (Kroekenstoel *et al*, 2006; Bhatnagar *et al*, 2010). Kroekenstoel *et al* (2006) noted that while the DHWA became more acute the dorsal parietal angle (DPA) became more upright and suggested that other factors such as bone absorption and bone remodelling may be involved. They do not appear to have considered that integument depth was a factor in the lack of parallelism between the DHWA and the DPA. The epidermal hoof thickens during maturation proximodistally, therefore throughout this period there must be a difference in integument depth. *In utero* the hoof wall is produced and thickens axioabaxially and *post partum* growth begins and continues throughout life, proximodistally from generation at the *stratum germinavatum*. The trigger to alter the source and direction of growth is not known but changing from *in utero* non-weight-bearing to *post partum* weight-bearing may activate this adjustment. In this present study the DPA did not become more upright with aging but continued to become more acute. This may be due to breed differences as Kroekenstoel *et al* (2006) studied Warmblood foals. In the study reported here the decrease in the DHWA was greater than the decrease in DPA and therefore these two angles still matured towards the parallelism seen in fully grown horses (Smith, 2004). There would appear to be no advantage to the young horse of the differing axes of the phalanges and HPA. It is possible that in the long legged Thoroughbred the difference is more obvious than in other breeds. Thoroughbred stud farms view a negative HPA in neonates as natural and are only concerned when there is not quick progression, over a few days to a straight or positive HPA.

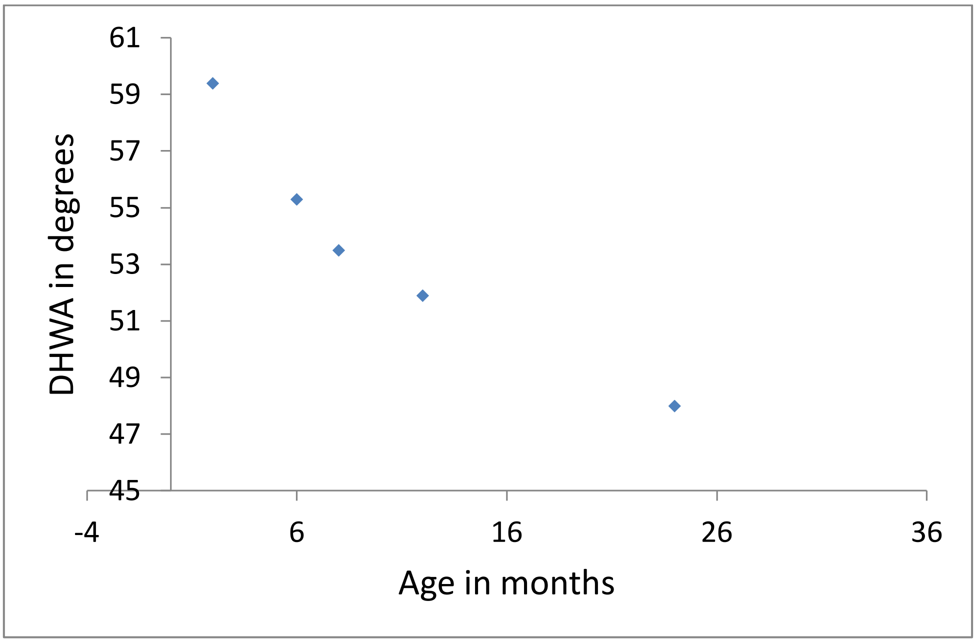


Figure 7.2: *Quadratic regression of DHWA data combined from Anderson and* McIlwraith *(2004) and from the study in Chapter 4 in this thesis;* R^2 *= 98.39%; P=0.008.*

7.2.3: *Integument depth*

Foals double their birth weight by 30 days and treble it by 90 days (Hintz *et al*, 1979; Nauwelarts *et al*, 2013). The weight of a mature TB is approximately 550kgs and the integument depth 16.1±1.75mm (Cripps and Eustace, 1999). Therefore at this time of rapid growth, it could be assumed that hoof wall integument depth would also increase and this was the finding in Chapter 4. The epidermis, *post partum*, is generated from the coronary band and grows distally; therefore any increase of the epidermal integument from new horn can only take place proximodistally. The proximodistal thickening of the integument causes this lack of parallelism between the DHWA and the distal phalanx. As the thicker hoof wall moves distally this projects the toe anteriorly and may also be a factor in the DHWA becoming more acute during maturation.

7.2.4: *Hoof shape*

In a mature horse hoof growth is occurring proximodistally in an obliquely truncated cone shaped hoof capsule (Kaspasi and Gosline, 1996); in the fetus and paediatric foal the cone was described as inverted (Bidwell and Bowker, 2006). Personal observations suggest that this is partially true but that the hoof capsule appears as a truncated oblique cylinder at birth, which then becomes an inverted cone when the coronary band expands and the epidermis thickens. The effect of the hoof wall thickening proximodistally can be seen in many foals, creating the inverted cone shape (Figure 7:3). This inverted cone shape makes evident the fact that foals, at three months of age are still supporting their body-weight on hooves developed *in utero*, with a small bearing area, when they are weighing more than three times their birth-weight. This imbalance between solar bearing area and weight may be overcome by a fast hoof growth rate but it does not seem that this fast rate is stimulated by an increase in pressure as it slows during the paediatric age.

Figure 7.3: *A dorsal view of a foal's digit at approximately three months of age; the hoof capsule shape is a truncated inverse cone; in some foals this is more apparent.*

7.2.5: *Bone growth*

Long bones increase in length and diameter during growth and changes in the relationship seen in this study between the angles; DPA, SPA and SBA may be partially due to circumferential bone thickening and contribute to the increased phalangeal alignment (Zoetis *et al*, 2003). These changes may be the result of bone scaling due to changes in stresses brought about by strenuous activity (Biewener, 1989). The bones of the foreleg are not only lengthening and thickening but also changing shape during maturation and this is exemplified by changes to the distal phalanx (Figure 7.4). Factors, other than changes in the hoof integument and bone growth, could also affect DHWA and HPA. These may include differential hoof growth rates at sites around the coronary band or compression of horn at the heel or dorsum. Some of these factors were investigated in Chapters 5 and 6 and will be discussed later in this chapter. The hoof capsule of a paediatric foal must respond to the increase in bone size, weight of foal, and changing phalangeal angles and may achieve this by rapid hoof growth and hoof renewal (Curtis *et al*, 2014).

Figure 7.4: *The developing distal phalanx changes in shape and size; from left to right, 38 days prior to birth, at birth, at four months.*

7.2.6: *Hoof width*

In June and November, where pairs of feet were uneven in width the correlation with the narrower hoof being steeper was not significant, contradicting a commonly held belief and the finding of Wilson *et al* (2009) in mature horses. It is possible that differences in hoof widths, which could not be established in this study, take time to respond to differences in DHWA. Foals appear to be born with symmetrical and paired front feet which remain so for many weeks (Bidwell and Bowker, 2006). As newborn Thoroughbred foals renew their hoof capsule in

145 days it would seem likely that any changes to hoof shape apart from wear and farriery, would take time to occur. The additional factors of differential hoof growth and plastic compression, discussed later, would probably also take time to occur.

7.2.7: *Conclusions drawn from this study (Chapter 4)*

Combining radiographic and hoof morphometric measurements in the same population group provided useful data regarding the relationships between the hoof capsule and the distal phalanx and also between the phalangeal and hoof pastern axes. Convergence of the HPA and phalangeal axes had not occurred by November, but appeared to be moving towards it. A factor in the non-alignment of the HPA and phalangeal axis, and lack of paralellism of the integument in foals, is caused by thickening of hoof integument proximodistally. Assumptions of phalangeal angles, similar to those used with mature horses based on external characteristics, cannot be made in foals. Anderson and McIlwraith (2004) measured DHWA from weanling phase, approximately six months. By starting the study reported here much earlier, at one to three months of age; a gap in our previous understanding has been filled by these original findings.

7.3: Chapter 5, Hoof growth and hoof compression

This chapter described one study documenting hoof renewal in newborn foals and a second combining hoof growth, hoof renewal and plastic compression in weanlings becoming yearlings.

7.3.1: Hoof renewal in newborn foals, study 1

The mean age at which the fetal hoof was removed, and therefore renewal occured was 145±15 days. Although the hoof renewal rate for foals being quicker than mature horses was anticipated, the rapidity surprised. The foot of a foal is smaller and therefore there is shorter distance for the hoof wall to grow from the proximal site of origin at the coronary band to the distal bearing border. Additionally it has been reported that foal hoof grows faster than mature horses (Butler and Hintz, 1977).

7.3.2: Foal hoof crease

The term ‘foal foot’ has been used to describe hoof distal to the foal hoof crease (FHC) (Ellis, 1998). This is a misnomer as the the hoof distal to the FHC has been produced *in utero* and should therefore more correctly be termed ‘fetal hoof’. The hoof proximal to the FHC, which is generated *post partum*, should be termed the ‘foal hoof’. The cause of the FHC is not known but may be a consequence of the foal changing from *in utero* non-weight-bearing to weight-bearing following birth. Another factor that may cause the FHC is the diet change of the foal associated with switching from nutients passed via the placenta to milk supplied by the mare and other feed such as grass taken orally (Huntingdon and Pollitt, 2005). In mature horses prominent growth rings have been associated with lameness and sudden changes in dorsal wall angulation (Dyson *et al*, 2011[b]).

Close observation of the FHC showed it to have a different appearence to common prominent hoof rings which regularly appear in the hoof wall, usually atributed to causes mentioned above. Smallwood (1989) described the FHC, which he found by radiographic imaging, in four Quarter Horse foals as a groove. Since a groove usually means a furrow from which material has been removed it was decided that this name was inaccurate. The observation that it appears more akin to a pleat or fold in the wall provided a need to rename it as the foal hoof crease. It is possible that the sudden weight-bearing after birth causes the distal phalanx to sink into the hoof capsule and in doing so temporarily alters the direction of hoof growth (Figure 7.5). This phenomenon has been observed in laminitis cases affecting mature horses, where distal sinking of the distal phalanx into the hoof capsule typically leaves a kink in the tubular structure of the *stratum medium* (Herthel and Hood, 1999).

Figure 7.5: *A preserved specimen of a three month old foal's hoof showing; left, the external hoof wall and the foal hoof crease; right, the internal structures, where the change in horn tubule direction is evident.*

In cases of partial hoof wall avulsion in horses, once the initial lesion has been treated and epithelialisation has begun it is useful to be able to predict the time that it will take for the new hoof generated from the coronary band to grow down to the bearing border. Sub-mural abscessation often erupts at the coronary band causing a horizontal crack in the hoof wall. The hoof wall will usually break when this crack nears the bearing border and again it is useful to calculate when this may occur in order for preventative strategies to be employed. Knowing the time that hoof renewal takes allows farriers, horse owners and veterinary surgeons to make an accurate prediction of healing time (Figure 7.6).

The limitations of this study resided in the methodology. As the trimming schedule was every 21 days this may mean that the accuracy of the data was ±10.5 days. However, as the study observed 150 foals and many were rejected where there was any doubt as to their suitibility, reducing the cohort to 45, then the results may have a greater accuracy than stated. This study drew attention to the need for further studies of hoof renewal and growth rates in older foals and yearlings which would be beneficial to make similar calculations. It was therefore decided to carry out a comparable study to the one reported here, using weanlings and additionally collecting data on hoof growth and plastic compression of horn.

Figure 7.6: *A partial hoof wall avulsion in a foal, caused by a sub-mural infection.*

7.3.3: Conclusion from study 1, Chapter 5

The discovery that newborn Thoroughbred foals renew their hooves replacing the fetal hoof in approximately half the time of mature horses had not been reported previously and was an original finding.

7.3.4: Hoof growth, hoof renewal and plastic compression in weanling/yearlings, study 2

This study investigated hoof growth in weanling/yearling Thoroughbreds to determine the time of hoof renewal and also whether hoof growth was influenced by plastic compression due to weight-bearing. In accordance with the hypotheses, hoof growth rate was slower compared to that of young foals; 0.25±0.06mm/day compared to 0.42mm/day (Smallwood *et al*, 1989), hoof renewal took longer at 283±29 days compared to newborn foals; 145±15 days (Curtis *et al,* 2014), and plastic compression of the hoof wall occurred over time. The hoof growth rate for weanling/yearlings, found in this study, was between the rates given by other authors for foals and mature horses (Smallwood *et al*, 1989; Reilly *et al*, 1998[b]). Faster hoof growth in equine juveniles may also be linked to the need for expansion of the hoof capsule during skeletal growth. As a herd animal, foals need to cover a comparable distance as the rest of the herd, so the relative loading due to locomotion would be similar. If however, the solar hoof and hoof wall thickness was proportionally narrower, then the pressure on the bearing surface of the hoof wall would be greater, which could require and therefore possibly stimulate faster hoof growth. This study did not record solar dimensions, hoof wall thickness or mass of the horses; future studies should include these variables, which may provide an answer as to why hoof growth rate is faster in maturing horses compared to adults. As reported earlier, other hoofed animals have similar rates of growth to horses in both young and mature animals (Geyer, 1979; Landerer, 1999; Stern, 2000). All of the hooves measured were longer at the end of the measurement period than the start. It is not known when the hoof capsule reaches its full extent but this study showed that it is still increasing at weanling/yearling age.

As anticipated, the time found for hoof renewal lay between that of newborn foals and mature horses and posed the question of why there would be any difference due to age? The distal phalanx in the horse is almost completely enclosed by the hoof capsule which, although flexible may constrict it. During a Thoroughbred’s growth from birth the weight may increase from 50–550kg (Thompson, 1995) and the phalangeal bones increase in size, so rapid hoof renewal may be a method of allowing bone expansion to occur (Zoetis *et al*, 2003). By rapid hoof growth and renewal the hoof capsule, though not shed, is being removed and replaced by a larger one which is analogous with a growing snake shedding its skin. The hoof capsule also changes shape at the age this study was undertaken, as previously proposed, from cylinder to inverted cone and finally to cone (Figure 7.7). Although the process of shape change is not understood, this may be part of the development that allows a wider distal phalanx to lodge within it (Bidwell and Bowker, 2006; Curtis *et al*, 2014).

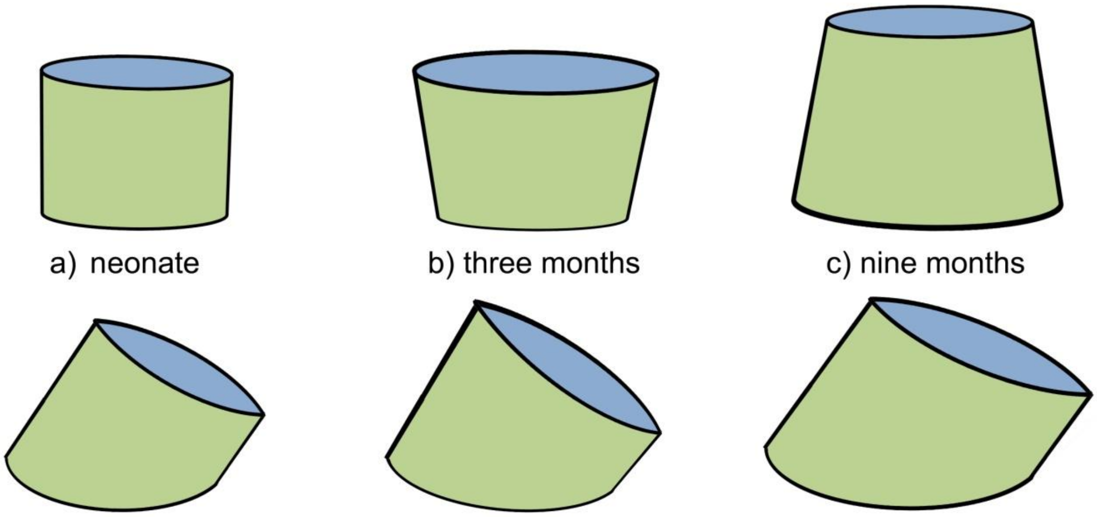


Figure 7.7: *The changing shape of a foal hoof capsule from left to right;* a*) truncated oblique cylinder at birth,* b*) truncated oblique inverted cone at approximately three months,* c*) truncated oblique cone by nine months.*

7.3.5: Hoof compression

The result that hoof horn compresses during growth is an original finding. The material of the hoof capsule has previously been described as viscoelastic and has been shown to respond to different weight-bearing phases by changing shape (Burn and Brockingham, 2001; Yoshihara *et al*, 2010). However, this is principally an elastic response and the hoof recoils to return to its typical shape at either standing or non-weight-bearing. It has previously been observed that hooves change shape, permanently or semi-permanently, in response to conformation or lameness and this has been described as plastic deformation (Dyson *et al*, 2011[b]). As a viscoelastic material it is possible that creep, which is a gradual change in length due to constant loading, occurs in hooves from the moment the foal stands, with uneven weight-bearing during standing causing alterations in shape. Additionally, plastic deformation may occur due to repetitive loading beyond the compressive strength of the material, likely occurring under much higher locomotive loads. Certainly, Bidwell and Bowker (2006) suggested that stresses imposed upon the hoof wall may cause responsive changes at the microscopic level which explain the wide variety of hoof shapes (Figure 7.8). In addition, postural and loading preferences during standing have been linked to the development of uneven feet (Kroekenstoel *et al*, 2006; Moleman *et a*l, 2006; Van Heel *et al*, 2006), which would suggest that creep may occur over time causing plastic compression.

When calculating the hoof renewal of the horses in this study the hoof compression rate for each hoof was used in predicting the time for both the proximal and distal unmeasured hoof to descend to a point in the wall. Previous studies of hoof growth have not accounted for hoof compression and this may mean that their results are affected. Where results in other studies have shown that hoof growth differs at different sites around the hoof wall it is possible that hoof generation, at the *stratum germinavatum*, is the same but differing compression accounts for apparent growth differences and therefore their findings (Faramarzi *et al*, 2009). In cases such as club foot there has been an assumption that the heels are growing faster than the toe but it is possible that the toe is compressing more (O'Grady, 2012). Alternatively, it is possible that it is multi-factorial and differing rates of growth and differing compression play a part in altering a well conformed hoof capsule to one that is described as unbalanced or deformed.

Figure 7.8: *Differential hoof growth in a foal's foot can be recognised by the varying distances from the coronary band to individual hoof rings.*

When farriers and horse owners complain that a horse does not have enough hoof wall growth; the focus is on how to increase hoof growth. Previous authors have suggested a number of factors affecting hoof growth including, season, sex, age, and diet (Ott and Johnson, 2001; Frackowiak and Komosa, 2006). This study took place from mid-December until mid-June, mainly in the winter and spring months and this may have affected the results. The two stud farms where the weanling/yearlings were stabled were professional organisations, feeding well balanced diets and producing high quality racehorses.

The findings clearly indicated that plastic compression of hoof horn does occur over time. This may lead to greater understanding of hoof deformity and direct farriery towards solutions using this knowledge. It is probable that many farriers have long recognised the effect of hoof compression without defining it as such. Terms such as “crushed heels” have a currency although it is not always clear whether this refers to under-run heels where the main cause of the hoof distortion would appear to be horn tubule bending, or low heels. Success has been claimed in treating low heels with the purpose of reducing weight-bearing by spreading loading by means of trimming and/or transferring weight away from the heels through the frog to promote hoof growth (Curtis, 2002; Milner and Hughes, 2012). It is possible that generation of horn at the *stratum germinavatum* and hoof growth rate remains unchanged but compression of horn is reduced, thereby causing an apparent increase in hoof wall growth at the heel. Strategies for improving hoof capsule distortion could be formed using this knowledge. Further discussion on this topic is after the findings in Chapter 6 are considered.

7.3.6: Conclusions from study 2 Chapter 5

This study showed that hoof growth rate in weanling/yearlings was slower and that hoof renewal took longer than in young foals. Using a novel technique to measure hoof growth allowed more sophisticated calculations which showed that hoof undergoes plastic compression during hoof wall decent. This was an original finding and future studies in this field will need to consider the effect that hoof compression has upon measurement.

7.4: Chapter 6, the effect of weight-bearing and regional solar loading upon hoof growth in foals

The study in Chapter 6 investigated hoof growth, dorsal hoof wall angle, hoof compression and solar hoof loading in paediatric foals. In accordance with the hypotheses, hoof growth rate (HGR) differed around the hoof wall and HGR was associated with loading; foals with an acquired flexural deformity of the distal interphalangeal joint (AFDdipj) differed in loading and regional HGR from the control group of healthy foals; dorsal hoof wall angle (DHWA) was less in the control group compared to the acquired flexural deformity group (AFD) group.

7.4.1: *Hoof growth rate*

The mean overall HGR did not differ between the control group and the AFD group; both were growing more rapidly than the weanling/yearlings in Chapter 5, and mature Lipizzaner horses measured by Josseck *et al* (1995). The results for both groups were similar to the HGR for American Quarter Horse foals, found by Smallwood (1989). There were significant differences in HGR at the three sites measured both intra-foot and inter-foot. In the control group the lateral hoof wall grew at a significantly greater rate, compared to the dorsal and medial sites. This may be a partial explanation for the asymmetrical solar shape of mature hooves where an imaginary dorsopalmar line bisecting the frog, would show the hoof capsule to be wider laterally (Roland *et al,* 2003); and also the difference between medial and lateral hoof wall angles, where the hoof wall at the lateral quarter is more oblique than at the medial quarter (Faramarzi *et al*, 2009).

This study was unable for practical reasons to measure heel growth because of the risk of injury to the foot and speed of renewal at the heel, therefore the most caudal area to be measured was the widest part of the foot. The overall HGR of the control and AFD groups did not differ but the intra-foot regional HGRs were significantly different. The foals with a flexural deformity showed a pattern of hoof growth in both limbs where the medial and lateral HGR did not differ but the dorsal HGR was significantly less. There has been an assumption within the equine industry that club foot is the result of growth at the heel being greater than at the dorsum (O’Grady, 2012). These results imply that a reduction of hoof growth at the dorsum is a causal factor in club foot.

7.4.2: *Dorsal hoof wall angle*

The DHWAs did not differ between left front (LF) and right front (RF) in either group but differed between groups. The foals comprising the AFD group were selected visually during routine farrier assessments. This would appear to show that an experienced farrier can determine cases of AFDdipj by assessing hoof angle and stance. Redden (2003) described a method of assessing club feet where a three degree difference in DHWA between the front feet can be repeatedly recognised and used to differentiate healthy from club foot. Clinicians recognise the stilted stance and gait of a foal with a flexural deformity which is almost impossible to define. Visual recognition of AFDdipj in paediatric foals involves inspection on a firm level surface to see if the heels of the front hooves are contacting the ground (Owen, 1975; Curtis *et al*, 2012). A pressure mat, such as the one used in this study would enable clinicians to identify foals with a mild flexural deformity or one at an early stage, which was beneath the threshold of the human ability to recognise.

Principal component analysis drew attention to the counter relationship between hoof growth and loading and the secondary counter relationship between dorsal hoof angle and compression. Healthy foals appear to develop the recognised hoof capsule shape with a more upright medial wall and greater solar surface area laterally because they load more medially and this affects apparent hoof growth, as greater medial loading results in greater compression. The amount of horn generation at the *stratum germinavatum* may not differ along its border but once generated the keratinised horn may be compressed unevenly to effectively cause differential hoof growth. This differential hoof growth would continue in response to the horse's loading which in turn would be affected by its conformation. Conformation in mature horses is relatively stable but there are changes during aging and occasionally injury or disease causes sudden changes. Regular farriery at appropriate intervals could arrest and may reverse some of these changes.

7.4.3: Hoof generation and hoof growth

The studies described in Chapters 5 and 6 have demonstrated that there is a difference between generation of horn and hoof growth. Horn generation is the production of keratinocytes at the *stratum germinavatum* and hoof growth is the amount of hoof wall that arrives at the bearing border. Between generation and arrival at the bearing border the hoof wall may be subject to uneven loading which compresses the material unevenly causing apparent differential hoof growth and this has an effect upon hoof shape.

7.4.4: Hoof growth rate and compression

In Chapter 5 the effect upon hoof growth rate of compression was shown and the compression factor of each individual hoof in the study (n=12) was used to calculate that hoof's growth rate and renewal time. In Chapter 6 compression of horn was measured separately from hoof growth but using the same markers. It was possible that greater loading in a region encouraged horn generation but greater compression overcame this and therefore the measured resulting hoof growth was reduced where there was greater loading. In the control group, loading was greatest medially and here was the least hoof growth and in the AFD group loading was greatest dorsally and this region had the least hoof growth.

7.4.5: Differential hoof shape, HGR, loading

The hoof shape of foals with a flexural deformity would appear to differ from healthy foals because regional solar loading differs and that this influences differential hoof growth between the dorsal hoof wall and the more caudal hoof wall. The greatest difference between the two groups was the amount of loading and hoof compression at the dorsum. This was much greater in the AFD group and consequently compression of horn was greater, hoof growth at the dorsum was less, and the dorsal hoof wall angle steeper.

There was a pattern across both groups where the RF had greater loading, hoof compression, and angle at the dorsum than the LF. This may be associated with laterality, where the stance of horses tends to be extending the LF with the RF bearing greater weight (McGreevy and Rogers, 2004). A link between posture and uneven feet has been shown, whereby foals extend the same limb during grazing and spend long periods in this stance (van Heel *et al*, 2006). In the study reported here the foals stood more equally on a pressure mat and though the feet were not forcibly placed parallel and alongside each other, the width of the mat (32.5cm) meant that they were relatively closely aligned. The data still showed uneven dorsocaudal loading which may imply that other factors, such as myotendinous tension differences between the front limbs, also play a part in differential loading.

Dorsal loading was greater than caudal or lateral in the RF of the control group and in the AFD group the difference was also greater in the RF. This suggests that it is typical for foals to bear weight differently left to right whether healthy or with a flexural deformity. Laterality and lateralised behaviour has been shown in both foals and mature horses and flexural deformities causing club foot has been associated with the RF in foals (McGreevy and Rogers, 2004; van Heel *et al*, 2006; Curtis *et al,* 2012). Wiggers *et al* (2014) showed that where dynamic differences were associated with uneven feet, it was the differences in DHWA, rather than the individual DHWA, that was significant. This may suggest that it is unreasonable to expect horses to have symmetry between hooves left to right in the front limbs and therefore radical attempts to correct this perceived fault may lead to undesirable consequences. There may be a conflict between aesthetic requirements of the owner and biomechanical requirements for the horse.

7.4.6: Implications for farriery

The information from this study that loading influences hoof growth may be used beneficially to shift loading in a way that produces more even growth around the hoof capsule and it is very possible that farriers already do this intuitively. Induced steeper dorsal walls, in mature horses, have been associated with slower hoof growth (Glade and Salzman, 1985). Trimming and shoeing techniques to alter weight-bearing and loading may have developed through custom, trial and error over hundreds of years without a scientific rationale. This does not mean that they are inappropriate but it may mean that with a greater understanding of hoof compression farriery can become more directed and evidence based.

In this present study the AFD group were not induced nor had their hooves trimmed to produce a steeper angle, in fact they were treated for AFD by trimming heels and complete stable rest for a period of one to two weeks and in two cases had Toe Extension shoes applied. Every effort was made to reduce DHWA but it is possible that while a Toe Extension shoe may treat the AFD condition, it could be causing more hoof wall compression at the dorsum by moving the centre of pressure dorsally. However, once the shoes were removed greater weight-bearing in the caudal region was recorded.

7.4.7: Limitation of study

Because in this study two feet were stood on the pressure mat, individual centre of pressure (COP) could not be determined and therefore all loading data was compared from the geometric centre of data and this may not coincide with the actual COP for each individual foot.

7.4.8: Conclusions drawn from this study (Chapter 6)

This study showed a link between hoof shape, compression of horn and hoof growth associated with uneven hoof loading in foals. This is an original finding which partially explains the change from symmetrical feet seen in foals to the asymmetry seen in mature horses and also left to right asymmetry in hooves where conformation differs between limbs. Uneven hoof loading affects hoof compression and growth and contributes to shape change in both healthy foals and those with a flexural deformity.

7.5: Summary of thesis

This thesis has described aspects of the hoof wall of the Thoroughbred foal from fetus through to yearling. The hoof of the developing horse is not only growing more quickly than at any time in its life but also has to cope with the rapidly increasing body-weight, enlarging skeleton and changing conformation. At the same time the epidermal hoof wall is thickening and growth rate slowing, the hoof capsule is expanding in size expeditiously, while changing in shape from cylinder to inverted cone with a decreasing dorsal hoof wall angle. Individual hooves alter hoof growth and shape in response to the uneven loading caused by differing limb conformation and conditions such as an acquired flexural deformity. Finally the matured hoof capsule has developed into a shape which can be described as an oblique truncated cone with unique individual characteristics, influenced by many factors including weight-bearing and loading.

The studies detailed in this thesis reported many original findings, which increase our understanding of the development of this extraordinary organ, the equine foot and its highly adapted and responsive hoof wall. Compression of hoof horn, affecting hoof growth rates and hoof shape is a major original finding, which has not been reported in literature studying the hoof wall of horses or other hoofed mammals. This knowledge may lead to beneficial treatment strategies in all ages of horse. The findings in the microscopy study showed, for the first time, that many of the structural features of the hoof wall are symmetrical across the hoof capsule, unlike mature horses. Regional solar loading was measured exceptionally in paediatric foals and shown to differ between healthy foals and those with an acquired flexural deformity affecting the distal interphalangeal joint. Hoof renewal times for both new-born foals and yearlings were original findings which have a clinical use in predicting healing. The dorsal hoof wall angle, hoof pastern axis, and phalangeal axis had not been reported in paediatric Thoroughbred foals previously and the findings in this study have clinical implications which should improve visual assessment of conformation.

8.0: References

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9.0: Manufacturers' addresses

1. Nair; Church & Dwight UK, Ltd., Folkestone, Kent, CT19 6PG, UK.
2. Photoshop 7.0; Adobe Systems Incorporated; 345 Park Avenue, San Jose, CA 95110-2704
3. Sound-Eklin TuDR, BCF Technology Ltd., 3 Tailend Court, Starlaw Rd., Livingston, EH54 8TE, UK.
4. Dicom viewer, Osirix Technologies Inc., 2258 Hill Creek Way Suite 200, Marietta, GA 30062, USA.
5. Excel 2007 (version 12); Microsoft Corp., One Microsoft Way, Redmond, WA98052-6399, USA.
6. Minitab Ltd.; Progress Way Binley Industrial Estate, Coventry, CV3 2TE, UK.
7. Apple iPhone, Apple Inc., 1 Infinite Loop, Cupertino, CA95014, USA.
8. Jameg Glue, Barnby Moor Stables, Kennel Drive, Retford, DN22 8QU, UK.
9. Metron Hoof, Epona Tech, Creston, CA, USA.
10. RSscan Lab Ltd, & 5 Pegasus, Great Blakenham, Ipswich IP6 0LW, UK.
11. Nikon DS-F1c; Nikon Instruments Europe B.V., Burgerweeshuispad 101, 1076 ER Amsterdam, Netherlands.
12. ImageJ;imagej.nih.gov/ij/

Appendix A: Ethics, health and safety and participant information

Contents

AP/NEW/PR PROJECT REFERENCE NO.

APPLICATION TO ANIMAL PROJECTS COMMITTEE FOR APPROVAL OF RESEARCH PROJECT

This form should be completed for all **NEW*** applications for University research support and submitted to the Chair of the Animal Projects Committee. Please refer to the notes for guidance on completion of the form. If the project involves behavioural studies of wild mammals, of animals in zoos or of invertebrates in the field please use form AP/NEW/PR/2. If making any reference to any other project in Section 6 or Section 7, the Project Reference No. should be given.

*A new project includes those where there are changes to Section 4, Section 5 or Section 6.

If this application relates (amendment or sub-project) to an application which has previously been reviewed, and approved, by AP Committee, please supply the corresponding Project reference number(s) from your decision letter(s). _____

Section 1

Is the project

Undergraduate ☐

Postgraduate Taught ☐

Research X

Is this application for funding?

☐ **YES**

X NO

If Yes, provide outcome of bid, if known?

Section 2

(a) Is the work to be carried out under an existing Home Office Project Licence?

☐ **YES**

X NO

(If answer is YES – a copy of the existing approved project licence application to be attached and this application to be completed in respect of this specific study/sub-project)

Home Office Project Licence No (if applicable)

Home Office Project Licence Title (if applicable)

Home Office Project Licence Approved No of Animals (species per year)

(b) Does the project require a new Home Office Licence? ☐ **YES** ☐ **NO**

Section 3

Is there a Personal licence holder?

☐ **YES**

X NO

Who holds it?

Is there a Personal licence No.?

Personal Licence No. **Date of issue**

Section 4

Duration of Project 5 Years (maximum 5 years)

Proposed Start Date of Project Jan 2013 (Month/Year)

Proposed End Date of Project Jan 2018 (Month/Year)

Section 5

Title of Project

A Study of hoof structure and hoof growth in Thoroughbred foals

Section 6

Name of researcher and co-workers

Simon Curtis (PhD Student)

Dr Sarah Jane Hobbs (DoS)

Dr David Elphinstone (2nd supervisor)

Dr Jamie Martin (2nd supervisor)

Section 7

Aims and Objectives of Project (Layperson's terms)

Study 1: Hoof growth

Aim 1: To investigate hoof growth in Thoroughbred foals.

Objective 1: To measure the typical hoof growth of TB foals and to investigate whether the rate of hoof growth changes with age using photographic measurements.

Aim 2: To determine whether the hoof compresses after generation from the coronary band.

Objective 2: To measure hoof growth at the toe and lateral hoof wall at regular time intervals as it grows towards the ground.

Aim 3: To investigate whether in cases of club foot the hoof growth will not be as quick at the toe compared to other sites on the hoof capsule and that the hoof growth rate will vary between front feet.

Objective 3: To measure the hoof growth of both front feet in a number of visually identified foals with unilateral club foot.

Aim 4: To determine the weight bearing pattern under the hoof during growth.

Objective 4: To measure the pressure pattern under the hoof of both forefeet during quiet standing.

Study 2: Hoof structure

Aim 1: To investigate the nature of the hoof growth ring (foal hoof crease) seen on Thoroughbred foals' hooves.

Objective 1: To establish the architecture of the foal hoof crease (FHC) by microscopic measurement.

Aim 2: To compare the hoof wall above and below the FHC.

Objective 2: To measure and compare the hoof wall material from in utero foals (non-weightbearing) to hoof grown after birth (weightbearing).

Aim 3: To compare the lamella (the structure attaching the third phalanx to the hoof wall) above and below the FHC.

Objective 3: To measure and compare the lamella from in utero foals (non-weightbearing) to hoof grown after birth (weightbearing)

Section 8

In layperson's terms explain precisely what will happen to the animal(s);

Study 1:

Before collection of data, permission will be gained from the stud farm owners of the horses used in the study using appropriate consent forms (attached). The horses will be checked for health. The work will take place at various Newmarket studs and will involve indoor work. The researcher, a farrier, involved in the study is experienced with Thoroughbred foals, horses and stud farms (his CV and biography is attached).

All equipment and material (Dremel, acrylic glue) used will be checked by the researcher prior to data collection. Stud management and staff will be briefed prior to the study of their role, the reasons for the study and safety issues. Only staff who have previously held foals for farriery work will be used.

Habituation

All mares and foals used in the study will be familiar with handling and their hooves being picked up by a farrier. Any sign of agitation or dangerous behaviour, will lead to them being removed from the area and monitored by one of the staff until resuming normal behaviour. This will mean that the foal will be removed from the study. Negative behaviours are described below:

Ears pinned back, head raised, head turned, head tossed, head shaken, head down, excessive defecation (Kaiser *et al*., 2006), threatening, ceasing forward movement, rearing, kicking, biting, lunging toward handlers, avoidance of handler and avoidance aids (McGreevy *et al*., 2009).

Methods;

Aim 1 will involve work with one foal to ensure all the equipment materials and method are working effectively and that the data can be collected. For this initial study the reference points on the foot are the naturally existing hairline of the proximal hoof and the foal hoof crease (FHC). The calibration of the photographs, using a circular sticker on the hoof wall, will be assessed using Metron-Hoof[1]. The study will continue using >30 TB foals, at various stud farms, once the equipment and methodology has been agreed.

Aim 2 requires a method of permanently marking a point in the hoof wall as the foal hoof crease grows in approximately three months after birth. A 2mm diameter mark will be made at a maximum 2mm depth with a Dremel tool and this will be filled with acrylic hoof repair glue. This leaves a circle of a different colour to the hoof wall which will grow out with the hoof and will be used as a reference. The hoof thickness of a foal is greater than 8mm and therefore this method does not penetrate the insensitive horn.

Aim 3 requires the same methods, materials and equipment as used in Aims 1 and 2 and 4.

Aim 4 requires the foals to stand square for a 20 second period on a pressure mat (an instrumented mat that is approximately 1 cm thick and 60 x 40 cm in size) which will be covered by a rubber mat (to protect the mat and reduce the possibility of a trip hazard). The mat will be positioned in a suitable flat part of a yard where the foals are more likely to stand quietly.

Confidentiality - Data collected from the study work will remain entirely confidential and anonymous. The information provided by the stud farm owners via consent forms ensures eligibility for taking part in the study. Information on the foals involved, as well as written consent, will be stored safely and securely and will not be available to any third parties. The stud farm owner will sign a consent form (attached) and receive a participant form.

Cut off point

In the unlikely event that a foal is used for 30 minutes, measurement will stop and the foal will be returned to its stable or paddock and it will be excluded from the study

Study 2:

Permission will be gained from the owners of dead foals used in the study with appropriate consent forms (attached) in the first instance. The foals will be screened for reasons of death and will be used for the study provided the reason is not expected to affect the data collected. Foals included in the study will have their front legs disarticulated at the fetlock joint in the post mortem (PM) room at Rossdale's Veterinary Practice, Newmarket as soon as possible after they arrive at the practice. They will be labelled and stored in a dedicated freezer. All dissection and preparation work will be carried out in the PM room at Rossdale's Veterinary Practice. The researcher (PhD student) carrying out all of the dissection/preparation/data collection/disposal is experienced with Thoroughbred (TB) horses, foals and stud farms and has been trained in all procedures (use of the bandsaw in the PM room / data collection and disposal by Rossdale's Veterinary Practice staff and training in sample collection and slide preparation by staff at the Free University of Berlin).

All equipment will be checked prior to each sample collection/preparation by the researcher. Laboratory staff at Rossdale's will be fully briefed about the study prior to commencement. Rossdale's have already verbally agreed to provide the assistance and resources for the study, based on gaining new knowledge from the work in the future.

Disposal of tissue: All waste tissue will be returned to the Jockey Club abattoir via their daily collection system where is will be disposed of according to their health and safety regulations.

Methods;

Aim 1, 2 and 3 will involve work with 1 necropsy hoof sample to ensure that all the equipment is operating correctly and that the methods are satisfactory to collect the data required. For this initial study the reference points on the foot are the naturally existing hairline of the proximal hoof and the foal hoof crease (FHC). Samples will be harvested at sites at, above and below the FHC to investigate the structure of the hoof wall and lamella. Once the methods are agreed from this trial the study will continue using a minimum of 30 TB necropsy foal and foetal hooves. These will be collected by the abattoir and delivered to the dedicated freezer at Rossdale's. The dead foal tissue will be selected based on an appropriate age for the study and having had no injuries or diseases that may have affected hoof growth before their death. Please note: No foals will be euthanized for this study. All foals will have died from other causes not related to the study and their tissue will only be used provided consent from the owners is given.

Confidentiality;

Data collected from the study will remain confidential and anonymous. The information provided by the Jockey Club abattoir via consent forms ensures eligibility for taking part.

Information about the foals involved, together with written consent will be stored safely and securely by the PhD student at his business premises in a locked filing cabinet and will not be given to any third parties. As the Jockey Club abattoir becomes the owner of the foals following their death, the abattoir manager will be required to sign a consent form to release the foal tissue for the study.

Section 9

a) How many, and which species of animals are intended to be used each year of the project

Year	No of animals	Species
Study 1:		
2013	**1**	**Thoroughbred foal (*Equus caballus*)**
2014	**30**	**Thoroughbred foal (*Equus caballus*)**
Study 2:		
2013/14	**30 minimum** **40 maximum**	**Thoroughbred foal (*Equus caballus*) - cadaver specimens that died of causes not related in any way to the study**

If animals are being killed/harmed complete Sections 9, 10, 11 and 12

Section 10

State any additional reasons that support this proposed use of animals to obtain the specific objectives. Is the number of animals you propose to use appropriate? – i.e. large enough to produce a satisfactory valid result and not greater, in accordance with the principles of Reduction, Refinement and Replacement.

Study 1:

Little is known about the growth of hoof horn: only two authors appear to have measured horn growth and these were for only 4 month periods on a total of 14 non-Thoroughbreds (Butler K D, 1997; Smallwood J E, 1989). Understanding the growth of horn will allow a prediction of the time taken for a hoof wall lesion to grow out. Club foot has been linked to an increase in lameness and a reduction in monetary value. Club foot can be a significant source of lameness (O'Grady S, 2012). A greater understanding of the physiology involved in the development of a club foot may lead to more rational and successful treatments. In a 4 year observational study (unpublished) Curtis identified that from 372 foals, 20% developed club foot. In order to investigate club foot in foals a total group size of 30 would give an estimated 6 club footed foals, so additional screening may be required to bring this number up to 10.

Study 2:

The opportunity to study hoof that has been developed *in utero* and *post partum* in the same foot only occurs in the first 4 months of a horse's life. Weight-bearing is considered to be one of the factors which causes change in the shape and morphology of equine hooves (Faramanzi *et al*., 2009). Most studies have focussed on mature horses and the effect of nutrition on horn growth (Reilly *et al*., 1998; Ott and Johnson, 2001). Understanding the effect of weight-bearing upon growth of horn may lead to understanding hoof deformities such as club foot, upright foot and collapsed heels in mature horses. Causes of hoof problems have been linked to the physiological and biochemical abnormalities of the hoof wall (Slater and Hood, 1997). Club foot has been linked to an increase in lameness and a reduction in monetary value. Club foot can also be a significant source of lameness (O'Grady, 2012). A greater understanding of the physiology involved in the development of club foot and other hoof deformities may lead to more rational and successful treatments.

Assessment of the potential for implementation of the 3Rs into a research protocol

For further information please see

http://www.nc3rs.org.uk/

and

http://www.homeoffice.gov.uk/comrace/animals/furtherinfo.html

Please use the following checklist to assist you in completing Sections 11, 12 and 13 of the application form.

If any answers require elaboration, please refer to the guidance notes for Sections 11, 12 and 13 as appropriate.

	YES	NO
Can the work be done without using animals or without using a project licence?		X
Have you considered any information relating to the 3Rs which allows you to select a replacement alternative to achieve some or all your	X	

objectives?		
Have you done a specific literature search to ensure that you have chosen the best methods for this research?	**X**	
Can existing replacement alternatives be adapted, or can you develop new alternatives, to accommodate your needs without compromising scientific integrity?		**X**
Can you modify your research strategy to allow you to use a replacement alternative for some stages of your work, without compromising your project? *Consider parallel animal/non-animal experiments for the validation of alternatives for your purposes; or* *Consider whether the results of experiments using replacement alternatives can improve any subsequent or complementary animal studies*		**X**
Where animal experiments appear to be inevitable, have you considered limiting the pain and suffering to the animals by implementing, reducing and refinement alternatives? *Consider:-* • *Use of less sentient species* • *Sharing animals* • *Using only one sex* • *Less invasive methodology* o *Injection of smaller volumes* o *Smaller needles* o *Less frequent sampling* • *Improving husbandry* • *Anaesthesia and analgesia* • *More-humane endpoints*	**X**	
Have you consulted A statistician The Named Vet The Named Animal Care and Welfare Officer?	**YES** **X**	**NO**

Section 11

Indicate the balance between the pain, suffering and distress to the animals involved and the likely benefits to be gained by the research.

Study 1:

The foals used in the study will continue with their normal lives. This is usually split between stabling and free exercise in a paddock with other mares and foals. The cost to the foal is therefore minimal as it will be asked to have its foot lifted as it is regularly used to doing. A behavioural assessment will be carried out as an additional measure of stress (as indicated in part 2). The foals and their mares chosen will be selected in the knowledge that they are likely to cooperate more readily.

A risk assessment form is attached.

Study 2:

As the foals died for reasons not related to the study prior to their use in the study this is not relevant.

Section 12

Are there ways in which the procedures could be refined to reduce the pain or suffering to animals without affecting the scientific validity of the project?

Study 1:

The study causes minimal cost to the horses involved as they will already be fully familiarised with their feet being trimmed and examined in a safe and enclosed environment. The stud staff are experienced at holding foals and their mares. The behaviour of the foals will be monitored throughout the measuring process and behaviour and health will be of utmost importance throughout the research work. These observations will be (primarily) carried out by the lead researcher. See attached risk assessment (Appendix 1).

There is no scope for reduction of the number of horses used in the study to gain useful data. The technique for marking the hoof is a refinement on previous authors, who have used a red hot brand to burn a permanent brand mark of indeterminate depth into the hoof, radiography, and one study where a radioisotope was used (Butler K D, 1977; Smallwood J E, 1989; Pollitt C C, 1990; Reilly J D, 1998).

The possible application of results gained from the study validates the work being carried out. The results will help to understand the hoof growth of foals. Ultimately this may lead to improvements in the area of hoof repair and treatments for club foot and laminitic conditions.

Study 2:

As the foals died for reasons not related to the study prior to their use in the study this is not relevant.

Section 13

Indicate what scope exists for reduction in the number of animals used and refinement in technique as the project progresses

Study 1:

The measurement of hoof growth can only be achieved by the use of live animals. Twenty randomly selected Thoroughbred foals is the minimum required to gain statistical information that can confidently be used in considerations of Thoroughbred foals in general. Selecting 10 visually identified club footed foals is the minimum to gain intra-individual correlations of hoof growth and comparisons with the randomly selected population. The technique has been refined so that it does not carry the risks of previous studies (Butler K D, 1977; Smallwood J E, 1989; Pollitt C C, 1990; Reilly J D, 1998). (Appendix 1)

Study 2:

Thirty necropsy hoof samples harvested from dead TB foals is expected to be the minimum number required to gain statistical information that can be confidently used to make judgements on the TB population as a whole. As this type of work has not been carried out before in this age range it is difficult to estimate how many are required to illustrate the change from *in utero* to 4 months of weight-bearing *post partum*.

Reference List

Butler K D, H. H. (1977). Effect of level of feed intake and gelatin supplementationon growth and quality of hoofs of ponies. *Journal of Animal Science* , 257-261.

Faramarzi, B, *et al*. (2009). Changes in growth of the hoof wall and hoof morphology in response to regular periods of trotting exercise in Standardbreds. *Am J Vet Res*, 1354-1364.

Kaiser, L., Heleski, C. M., Siegford, J. And Smith, K. A. (2006) Stress related behaviours among horses used in therapeutic riding program. *Journal of the American Veterinary Medical Association*. 228 (1) 39-45

McGreevy, P. D., Oddie, C., Burton, F. L. And McLean, A. N. (2009) The horse–human dyad: Can we align horse training and handling activities with the equid social ethogram? *The Veterinary Journal*. 181 (1) 12-1

O'Grady, S. (2012). Flexural deformities of the distal interphalangeal joint (clubfeet). *Equine Veterinary Education* , 1-9.

Ott and Johnson (2001). Effect of trace mineral proteinates on growth and skeletal and hoof development in yearling horses. *J Equine Vet Sci*. 21, 287-291.

Pollitt C C. (1990). An autoradiographic study of equine hoof growth. *Equine Veterinary Journal,* 366-368.

Reilly J D, C. D. (1998). Effect of supplementary dietry biotin on hoof growth and hoof growth ratein ponies: a controlled trial. *The Equine Hoof, Equine Veterinary Journal Supplement , 26*, 51-57.

Slater and Hood (1997). A cross-sectional epidemiological study of equine hoof wall problems and associated factors. *Equine Vet J.* 67-69.

Smallwood J E, A. S. (1989). A xeroradiographic study of the developing equine foredigit and metacarpophalangeal region from birth to six months of age. *Veterinary Radiology , 30* (3), 98-110.

Materials

1. Metron-Hoof, EponaTech LLC, P.O. Box 361, Creston, CA 93432

Section 14 **List hazardous chemical substances that will be used and expected quantities (e.g. those classified under the CHIP Regulations as toxic, harmful, corrosive, irritant, flammable, oxidising, dangerous for the environment).**		
Ethanol	Xylene	
Formaldehyde		
Please attach COSHH assessments for the above mentioned substances.		

List hazardous biological agents that will be used and expected quantities.	
Please attach COSHH assessments for the above mentioned substances.	

Details of equipment used.	
Band saw	Microtome
Dremmel tool	Pressure mat
Please attach a risk assessment for the operation of any equipment that exposes persons to a significant hazard (e.g. cut, trap, nip/crush, electrocution,	

entanglement, very hot / very cold etc).

Details of animal handling operations. List precautions that will be taken to prevent injuries being sustained during contact with animals (e.g. kick, bite, crush, poison etc).
Foals will be handled by experienced stud staff and farrier only. Study 1 does not involve any additional experiences beyond that which the mares and foals would routinely have.
Please attach relevant risk assessment. Attached

Details of specific hygiene or containment requirements. (e.g. animal allergen, infection risk).
Stud site health and safety procedures will be followed at all times by the farrier and research staff entering and leaving the site.
Have any training needs specific to this project been identified that must be undertaken prior to the start of the project? If so give details as to nature of training and delivery and assessment methods.
☐ **YES X NO**
All relevant training has already been carried out by the farrier, and PhD student undertaking the work. One researcher (DoS) will accompany the farrier to operate the pressure mat, who is already trained in the use of pressure mat for data collection with horses. This researcher is not allergic to horses.

Is Health Screening necessary for those carrying out this project? If so give details.
☐ **YES X NO**
All personnel involved in the project will not be asked to carry out any tasks beyond their normal working duties.

Section 15 **Has approval been given by the Ethics Committee of any collaborating institution?**	☐ **YES** ☐ **NO**
N/A **Please attach a copy of the relevant documentation relating to external approval.**	

Signature/Name of Principal Investigator Dr Sarah Jane Hobbs

Date 5th November 2012

The form should be emailed/sent to Louise Price, Committee Secretary, at least two weeks prior to the Committee meeting.

Applications can be emailed to lmprice1@uclan.ac.uk or posted to Graduate Research School, Room 12, Greenbank Building

Notes for Guidance on completion of application form

Projects that must be considered by the APC are ones which involve

- handling of and interaction with all animals including those protected under the 1986 Act[1]
- direct intervention with animals
- observational studies that may cause distress*

and would normally complete Form AP/NEW/PR

Animal studies which do not fall into the above categories e.g. analysis of ongoing or historic data and those based on questionnaires do not need to be considered by the Committee.

Project Reference No: All projects are allocated a reference number when submitted to the Committee. This reference number should be quoted on all correspondence/project forms e.g. closure report, renewal form

Section 1

Tick relevant box and, if yes, provide outcome of bid, if known.

Section 2

Tick relevant box and include Project Licence Number; Title and Approved No of Animals

Section 3

Tick relevant box and include name of licence holder, personal licence No. and date of issue

Section 4

Indicate the number of years (maximum 5) the project will be running together with the proposed Start Date and End Date of the Project (Month/Year)

Section 5

This should be the definitive title of the project

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[1] The Act regulates scientific procedures which may cause pain, suffering, distress or lasting harm to "protected animals"; it refers to these as "regulated procedures". "Protected animals" are defined in the Act as all living vertebrate animals, except man, as well as one invertebrate species, the common octopus. The definition includes foetal, larval and embryonic forms which have reached specified stages of development.

Section 6

All the names of research workers and co-workers should be included. If there are any external collaborators indicate the name of the Institution(s)

Section 7

The aims and objectives of the project should be made very clear as there are external members on the Committee.

Where available more detailed information should be attached to the application. This section should be no more than 1000 words.

Section 8

You should make clear all procedures to which the animals will be subjected to, include any adverse effect to the animals e.g. tissues collected from abattoir and analysed for zymogen, induction of diabetes for *in vitro* study.

Section 9

Include any justification (e.g. numbers required for statistical analysis) of the numbers of animals to be used each year of the project. Where more than one species is to be used, indicate how many of each. If animals are being shared please complete Section 10-12.

Section 10

Give any additional reasons that support the proposed use of animals to obtain the specific objectives. State the statistical methods you would use to analyse your results and the statistical basis of your estimation of the number of animals required.

Section 11

Indicate the balance between the pain, suffering & distress to the animals involved and the likely benefits to be gained by the research.

Section 12

If the answer is YES information is required as to why this is not done. **It is expected that an explanation will be given in this section.**

Section 13

Give details of what scope exists for the reduction in the number of animals used and refinement in technique as the project progresses.

Section 14

Give details relating to Health and Safety aspects. Has the person received training on killing or is deemed competent?

Section 15

If there are any External Collaborators in Section 5 has approval been given by the Ethics Committee in the Institution(s). If the answer is **YES** provide relevant documentation.

Principal Investigator should sign and date the form or type in name if submitted electronically.

Submission of Project Application Form

The Animal Projects Committee normally meets four times a year. Project Application forms and all supporting documents should be with the Secretary no later than two weeks before the date of the meeting.

If the project needs to be approved immediately this should be forwarded to the Secretary and the Chair of the Committee.

Reference Documents

http://www.nc3rs.org.uk

http://www.homeoffice.gov.uk/comrace/animals/furtherinfo.html

www.hse.gov.uk

Ethical approval notifications

Ethical approval, Myerscough College

Elphinstone, David <delphinstone@myerscough.ac.uk>
to me

Hi Simon,

Good to hear from you – sorry I did not have much chance to speak at the Farriery conference.

Copies of the documents you submitted and information regarding approval:

Simon Curtis	Approved 25-01-12	5	JR	11/AN/75

David

Myerscough College
Dr David Elphinstone
Director of Research
Bilsborrow, Preston, Lancashire, PR3 0RY
Tel: 01995 642222 Direct line: 01995 642309 Fax: 01995 642333
Web: http://www.myerscough.ac.uk/

From: Simon Curtis [mailto:curtisfarrier@gmail.com]
Sent: 18 January 2014 13:37
To: Elphinstone, David
Subject: Ethical Approval

Ethical approval, University of Central Lancashire

MEMORANDUM

To: Sarah Hobbs
Division of Sport, Exercise & Nutritional Sciences

From: Louise Price
Committee Secretary
Graduate Research School Office
☎ 3486
✉email: lmprice1@uclan.ac.uk

Date: 28th November 2012

Ref: RE/12/06/SH

Animal Projects

I can confirm that Chair's Action has now been taken and the following project has been approved.

- **A Study of Hoof Structure and Hoof Growth in Thoroughbred Foals**

Please note that this is also to notify you of the reference number allocated to your project (as stated above). Please use this reference number whenever your projects are returned to the Committee for renewal, closure etc.

Regards,

Louise Price

Participant information sheets

Microscopy Participant sheet, Chapter 3

<u>Participant Information Sheet – Equine Abattoir</u>

You have been invited to participate in a piece of equine research. The research will involve studying the hoof structure of foals. This form provides basic information regarding the testing as well as asking for your consent to participate in the study. If you have any questions which you feel are unanswered by the information provided below then please do not hesitate to me on the details provided at the end of this form.

The testing procedure
Study work will take place at Beaufort Cottage Laboratories. You will be asked to provide the hooves of dead foals for microscopic studies of the front feet. Your staff will deliver the foals' feet to the post mortem room freezer for studying by Simon Curtis.

Risks
You have been invited to take part in this study as you are a competent equine abattoir, employing experienced collectors of dead foals on a regular basis and have done plenty of this work previously. You and your staff will not be asked to do anything that you would not experience during your regular work.

Consent
Participation in the study is voluntary and you are able to withdraw at any time during any part of the testing. Once the testing is complete it will not be possible to remove specific foals data from the study (due to making all data anonymous), so it is important to make sure you have read this information and asked any questions before completion of testing.

Collected data
All data that is collected from your participation will be anonymous and results will be analysed and used to determine future research work. The information you provided before the testing at the laboratory and signature to agree to test is to ensure your safety and eligibility. Information you have provided will be safely and securely stored in a computer with a pass code.

If you are happy to go ahead with participating in the testing please sign the attached consent form. It is a requirement you provide a signature to reflect agreement to perform the research.

All communications should be made to,

Simon Curtis, The Forge, Moulton Rd, Newmarket, Suffolk, CB8 8DU

Tel: 01638 665761, Mobile: 07818991091

Email: SJCurtis@uclan.ac.uk or curtisfarrier@gmailcom

<u>Agreement to testing</u>

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of participant;

Print Name: ………………………………………………………………

Signature: ……………………………………………………………………..

Date: …….. /…….. /………….

Witness

Print Name: ………………………………………………………………

Signature: ……………………………………………………………………..

Date: …….. /…….. /………….

Participant Information Sheet – Stud Farm

You have been invited to participate in a piece of equine research. The research will involve measuring radiographic angles and hoof metrics of foals. This form provides basic information regarding the testing as well as asking for your consent to participate in the study. If you have any questions which you feel are unanswered by the information provided below then please do not hesitate to me on the details provided at the end of this form.

The testing procedure

Study work will take place on your stud farm and will inside the foal barn. You will be asked to provide your foals for hoof measurement and radiography of the front distal phalanges. Your staff will lead them to the prepared radiography area and hold them while the radiographic images are taken. Safety gowns will be worn by all in the vicinity of the radiography. The foals will be returned to their stable where their hooves will be measured by Simon Curtis (SJC).

Risks

A full and thorough risk assessment has been carried out by the vets involved and SJC to minimise any potential risks to you, the horses and the researchers. The main risk of taking part would be being kicked or bitten by the foals or their dams. You have been invited to take part in this study as you are a competent stud farm, employing experienced handlers who hold mares and foals on a regular basis and have done plenty of this work previously. You and your staff will not be asked to do anything that you would not experience during a normal veterinary radiographic investigation or farriery hoof trim.

Consent

Participation in the study is voluntary and you are able to withdraw at any time during any part of the testing. Once the testing is complete consent cannot be withdrawn so it is important to make sure you have read this information and asked any questions before completion of testing.

Collected data

All data that is collected from your participation will be anonymous and results will be analysed and used to determine future research work. The information you provided before the testing on the stud and signature to agree to test is to ensure your safety and eligibility. Information you have provided will be safely and securely stored in a locked filling cabinet.

Ethical Consent

Ethical consent for the study has been approved by the Myerscough College committee and the University of Central Lancashire; reference 5/JR/11/AN/75.

If you are happy to go ahead with participating in the testing please sign the attached consent form. It is a requirement you provide a signature to reflect agreement to perform the research.

All communications should be made to:
Simon Curtis; The Forge, Moulton Rd, Newmarket, Suffolk, CB8 8DU

Tel: 01638 665761
Email: curtis.farrier@talk21.com

Agreement to testing

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of participant;

Print Name and Stud: ……………………………………………………………………

Signature: …………………………………………………………………….

Date: …….. /…….. /………….

Witness

Print Name: ……………………………………………………………………

Signature: …………………………………………………………………….

Date: …….. /…….. /………….

Participant Information Sheet – Stud Farm

You have been invited to participate in a piece of equine research. The research will involve measuring hoof metrics of foals. This form provides basic information regarding the testing as well as asking for your consent to participate in the study. If you have any questions which you feel are unanswered by the information provided below then please do not hesitate to me on the details provided at the end of this form.

The testing procedure
Study work will take place on your stud farm. You will be asked to provide your foals for hoof measurement of the front feet. Your staff will hold the foals while their hooves are marked and measured by Simon Curtis (SJC).

Risks
A full and thorough risk assessment has been carried out by SJC to minimise any potential risks to you, the horses and the researchers. The main risk of taking part would be being kicked, bitten, or jumped on by the foals or their dams. You have been invited to take part in this study as you are a competent stud farm, employing experienced handlers who hold mares and foals on a regular basis and have done plenty of this work previously. You and your staff will not be asked to do anything that you would not experience during a normal veterinary foot investigation or farriery hoof trim.

Consent
Participation in the study is voluntary and you are able to withdraw at any time during any part of the testing. Once the testing is complete data cannot be withdrawn (as it will be anonymous) so it is important to make sure you have read this information and asked any questions before completion of testing.

Collected data
All data that is collected from your participation will be anonymous and results will be analysed and used to determine future research work. The information you provided before the testing on the stud and signature to agree to test is to ensure your safety and eligibility. Information you have provided will be safely and securely stored in a computer with a pass code.

If you are happy to go ahead with participating in the testing please sign the attached consent form. It is a requirement you provide a signature to reflect agreement to perform the research.

All communications should be made to,

Simon Curtis, The Forge, Moulton Rd, Newmarket, Suffolk, CB8 8DU

Tel: 01638 665761, Mobile: 07818991091

Email: SJCurtis@uclan.ac.uk or curtisfarrier@gmailcom

Agreement to testing

I understand the risks associated with this study and that all the data produced will be treated with confidentiality and individually. If I wish, the results produced will be available to me.

I willingly agree to participate in the current study. I have read the above information and understand that withdrawal from the study is possible until all data has been collected.

Name of participant;

Print Name and Stud: ……………………………………………………………………

Signature: …………………………………………………………………….

Date: …….. /…….. /………….

Witness

Print Name: ……………………………………………………………………

Signature: …………………………………………………………………….

Date: …….. /…….. /………….

Risk assessment

Risk assessment, Chapter 3

Risk Assessment for Microscopic Hoof Study version 1

Beaufort Cottage Laboratories, Newmarket**,** CB8 8JS

Identified hazards:

1. Using a band saw to harvest hoof sections from necropsy samples.
2. Using chemicals to prepare slides of hoof samples.
3. Using a microtome to produce fine slices of hoof samples.

At risk:

1. Laboratory staff
2. The researcher.

Evaluated risks and precautions:

. **Risk**	**Precaution**
2. A band-saw can inflict a severe cut to a hand or arm.	The researcher has received training and is experienced in their use. Only the researcher will use the band-saw. The band-saw guard will be used correctly.
1 & 2. Alcohol is used in slide preparation. Inhalation may cause irritation of nose and throat, eye exposure can cause stinging and lacrimation.	The alcohol will be used in minimum amounts by the researcher and laboratory technician. Eye protection will be worn. Both have been trained in its use and safety procedures. At any sign of eye or throat irritation the person will leave the area and receive treatment. (Appendix 2, P1)
1 & 2. formalin (dilute formaldehyde) is used in slide preparations. Irritation of nose and throat, eye exposure can cause stinging and lacrimation. Long term high exposure can cause serious Illness.	Formalin will be used in minimum amounts by the researcher and laboratory technician. Eye protection will be worn. Both have been trained in its use and safety procedures. At any sign of eye or throat irritation the person will leave the area and receive treatment. (Appendix 2, P2-4)
1 & 2. Xylol (Xylene) is a solvent used In the preparation of slides. It may be harmful if swallowed and if In contact with eyes.	Xylol will be used in minimum amounts by the researcher and laboratory technician. Eye protection will be worn. Both have been trained in its use and safety procedures. At any sign of eye irritation the person will have their eyes rinsed with water. Where this is not immediately effective then medical treatment will be sought. (Appendix 2, P 5)
2. A Microtome can inflict a severe cut to a hand when cleaning the blade.	The researcher has been trained to use the Microtome and clean the blade. The blade is only cleaned downwards and never across it.

In General:

Before using any of the cutting machines (band-saw and microtome) they will be checked to see that they are in good working order. No one who has not been trained in their use will be allowed in the vicinity. Cleaning procedures will only be carried out by the researcher. Beaufort Cottage Laboratories has its own written health and safety policy (available upon request) which will be adhered to at all times during the collection, preparation and disposal of hoof samples.

Risk assessment, Chapter 4

MYERSCOUGH COLLEGE

A study of the hoof metrics and phalangeal angles in Thoroughbred foals	**PROGRAMME AREA** **Three Newmarket Stud Farms**	ASSESSMENT UNDERTAKEN Signed: SJ Curtis Date:	ASSESSMENT REVIEW Date:

STEP ONE	STEP TWO	STEP THREE
List significant hazards here:	*List groups of people who are at risk from the significant hazards you have identified.*	
General handling of the horse includes risks of being knocked over, stood on kicked or bitten.	The researcher, foal and/or mare handler, and co-worker	All study researchers and participants have a history of handling horses and are experienced in being watchful of the horse in relation to themselves. Personal protective clothing will be worn at all times including sturdy footwear. The horses will be restrained by the use of a head collar or bridle during all of the procedures discussed here involving the horses.
holding foals includes risks of the horse kicking, biting, and rearing, self injection, adverse reaction.	The foal, handler and co-worker	All radiography will be carried out in a designated enclosed area. No electrical equipment will be left unattended during radiography and measuring. All electrical equipment will be checked that it is in good working order prior to use. Care will be taken to remove anybody from the vicinity not involved in the study and incorrectly gowned.
Radiography includes the risk of exposure to radiation, tripping or falling over wires. Also risk of electric shock.	The co-worker/s and handlers	A riding hat to current British safety standard (BS EN 1384 or PAS 015) and sturdy footwear must be worn by the person handling the horses The horses involved in the study have been hoof trimmed previously by the researcher and have shown normal behaviour. The process must be stopped immediately if the horse shows signs of significant stress, or the handlers decide it unsafe to continue with the

		process. This is unlikely because all horses used in the study will be used to being handled as part of their normal management routine.
Measuring the horse also involves risk to the handler from the horse becoming upset by the measuring process. Workers could be bitten, kicked or stood upon.	The researcher and handlers	
Positioning disc markers onto the dorsal hoof wall of the horse includes risk of being kicked, knocked over, bitten or stood on.	The handlers and co-workers	All handlers and co-workers participating in collecting data are experienced with handling horses. Researchers will be aware at all times of their position in relation to the horse. Personal protective clothing should be worn (sturdy boots, gloves and a riding hat to current safety standards PAS 015/BSEN 1384 or equivalent).
Standing the foal on 50mm radiographic wooden blocks carries a risk of being kicked, stood on and knocked over.	The co-workers	The researcher and co-workers participating in collecting data will have undergone safety training and be experienced with handling horses. The researcher and co-workers must be aware at all times of their position in relation to the horse. Personal protective clothing should be worn (sturdy boots.
General handling and moving of equipment includes risk of back injury as well as arm, hand, leg and foot injury if equipment is dropped.	he researcher and co-workers	Ensure correct manual handling techniques are known and used at all times when moving equipment such as jump wings and poles. If the item in question is considered too heavy it must be moved between two people to avoid injury. All researchers and co-workers will be aware of this.
The study includes risks of the researcher and co-workers, bumping	Horse, researcher and co-workers	The radiographic area will be set up before the mare and foal is brought in with extra attention paid to making sure that all wires and equipment used are positioned safely. All electrical equipment shall be placed as near as possible to the side of the area so that wires are not running across the horse's path. The site will have been risk assessed before hand to make sure that the area surface is level and in good condition and that researchers are aware of entrances and exits,

into or sliding on equipment used.		fire escapes and assembly points and first aid stations. No unauthorised persons shall be allowed into or around the testing area or near the equipment. Notices will be placed in the testing area to ensure this.
Electrical equipment could become faulty and cause injury.	The researcher, co-workers, and horses	All equipment will have been PAT tested and checked prior to use for loose wires or possible problems. Equipment which needs to be connected to a main power supply will have a circuit breaker attached.
The testing procedures includes the risk of slipping when inside or exiting the area due to the faecal material underfoot.	The researchers, co-workers, handlers	Be aware at all times of the surface being stepped on and take time to clean any excess build up of faeces of shoes whilst in and outside the area.
Zoonotic disease	Handler, researcher and co-workers	Hands washed and good hygiene will be expected by all personnel involved.
Allergy to horses	All personnel involved in the trials	All personnel involved in the trials will be aware that horses will be used in the study and the lead researcher will ensure that all members of the team are not allergic to horses. In the event of an allergic reaction occurring a first aider will be called (as above) and treatment will be suggested according to severity of reaction.

Risk assessment, Chapters 5 and 6

Risk Assessment for Foal Hoof Growth Study

[redacted] Stud, Newmarket**,** CB8 [redacted]

Identified hazards:

1. Foals and their mares (if present) kick, bite and react unpredictably to sudden stimuli.
2. Using electrical equipment has a potential danger.

At risk:

1. The foals and mares
2. Horse handlers.
3. The researcher.

Evaluated risks and precautions:

Risk	Precaution
1. The foals and mares becoming stressed or agitated.	The horses will be held by trained and experienced handlers. They will initially try to calm the mare and/or foal. If either remains stressed or agitated, they will be removed from the study and returned to their stable, where they will be monitored until calm.
1. The foals and mares injuring themselves by falling on hard ground or running away into objects.	The study will be carried out inside with bolted doors. Any potentially injurious objects will be removed from the area. The floor will have a soft bedding, e.g. straw or woodchip.
1. Marking the hoof with a battery operated Dremel tool. Danger to sensitive tissue, noise frightening the horses, cables to trip, electrocution.	A battery operated Dremel tool which has no dangerous cables and is quiet will be used. The chuck stop is fixed at 2mm depth, the hoof wall is >8mm in thickness (attached picture).
2&3. Foals or mare behaving dangerously, e.g. biting, kicking and/or standing on the handler or researcher.	Only those directly involved will be present. The horses will be held by trained and experienced handlers. Any mare or foal that is fractious will be removed from the study.
3. The foal or mare may kick or bite the researcher. The mare may kick or bite the researcher.	The researcher is experienced with holding a foal's foot for routine hoof trimming. The handlers will keep the mare at a safe distance from the researcher and not allow her to turn away. The handlers are used to holding a mare while its foal has its feet trimmed. Any mare or foal that is fractious will be removed from the study.

In General:

Before beginning the data collection the area will be assessed to see that there are no hazards in the vicinity, e.g. tool boxes, machinery, and that the area is secure with gates and doors shut. The horse handlers will be briefed on the procedure to measure and how they should hold the foal (and mare if present). There will be two handlers to the foal and one to hold the mare. The handlers will be asked about the foals and mares to ensure that they are healthy and well behaved. The stud farm management and all staff will be made aware that there is activity involving the researcher, staff and horses. The hoof wall is an insensitive structure, similar to a human finger nail but much thicker, and can therefore be marked without causing any discomfort to the foal, The mark will grow out in approximately four months.

Disposal of chemicals and tissue waste, Chapter 3

Email from Dr Alastair Foote,
Partner in charge of **Beaufort Cottage Laboratories**;
High Street,
Newmarket
Suffolk CB8 8JS

Alastair Foote <alastair.foote@rossdales.com> 2/26/13

to me

Hi Simon

Genta Medical (Unit 17D, Marston Business Park, Tockwith, York YO26 7Q) dispose of our xylene and any other dangerous chemicals.

Cannon Hygiene (www.cannonhygiene.com) dispose of our other clinical waste (including any remaining fixed tissues etc).

I hope that is sufficient for you.

Will catch up when you are back from the US.

All the best

Alastair

HAZARD DATA SHEET

CHEMICAL	**Alcohols, 50%, 70%, 90%**
SYNONYMS	Ethanol; Ethyl Alcohol
HANDLING	Wear protective clothing, gloves and eye protection. Do not breathe the vapour. Avoid prolonged or repeated exposures. Ensure adequate ventilation. Safety pipettes must be used for solutions.
INCOMPATIBILITIES	See Handbook of Reactive Chemical Hazards Can react vigorously or violently wih oxidising materials.
STORAGE	Avoid exposure to moisture. Store in tightly closed containers in a cool dry place above freezing point. Protect from sources of ignition. Secure chemicals from unauthorised use. Segregate stock.
MAJOR HAZARDS	Toxic by inhalation, in contact with skin and if swallowed.
FIRE EXTINGUISHER	Water spray, dry powder or carbon dioxide.
FIRST AID	EYES - Irrigate thoroughly with water for 15 minutes. If necessary seek medical attention. INHALATION - Remove person to fresh air. In severe cases or if exposure has been great, seek medical attention. SKIN - Wash area with large amounts of water for 10 minutes. Remove contaminated clothing. Unless minor incident seek medical attention. INGESTION - Wash out mouth thoroughly and give water to drink. DO NOT induce vomiting. Seek medical attention.
SPILLAGE / DISPOSAL	If local regulations permit, mop up with plenty of water and run to waste, diluting greatly with running water. Otherwise absorb on inert absorbent, transfer to container and, if volatile, transport to safe open area for atmospheric evaporation. Alternatively arrange for removal by disposal contractor. Ventilate area to dispel residual vapour.

HAZARD DATA SHEET

Product Name	Formaldehyde (concentrate phosphate buffered)
Characterisation	Solution in water
CAS Number	50-00-0
EEC Number	200-001-8
Components	Methanol 10% Formaldehyde 40%
First Aid	Eye contact: Irrigate thoroughly with water for at least 10 minutes. OBTAIN MEDICAL ATTENTION. Inhalation: Remove from exposure, rest and keep warm. In severe cases, or if exposure has been great, obtain medical attention. Skin contact: Drench skin thoroughly with water. Remove contaminated clothing and wash before re-use. Unless contact has been slight, OBTAIN MEDICAL ATTENTION. Ingestion: Wash out mouth thoroughly with water and give plenty of water to drink. OBTAIN MEDICAL ATTENTION.
Fire Hazards	Combustible
Firefighting	Water spray, dry powder or vaporising liquids
Spillage	Wear appropriate protective clothing. Inform other to keep at a safe distance. If local regulations permit mop up with plenty of water and run to waste, diluting greatly with running water. Otherwise absorb on an inert absorbent, transfer to container and arrange removal by disposal company. Ventilate area to dispel residual vapour. Wash site of spillage thoroughly with water and detergent. For large spillages liquids should be contained with sand or earth and both liquids and solids transferred to salvage containers. Any residues should be treated as for small spillages.
Storage	Store in a warm place (above 20°C) to prevent freezing. Keep well closed and protected from direct sunlight and moisture.
Protective Measures	As appropriate to quantity handled. Respirator: Self-contained breathing apparatus Ventilation: Fume cupboard, flameproof Gloves: Rubber or plastic

Formaldehyde hazard data sheet continued

Protective Measures	Eye Protection: Goggles or face-shield Other Precautions: Plastic apron, sleeves, boots - if handling large quantities
Form	liquid
Colour	colourless
Odour	pungent
Melting Point	n/a
Boiling Point	96°C
Density	1.8
Vapour Pressure	n/a
Vapour Density	n/a
pH value	3 - 4
Flash Point	62°C
Solubility in Water	Miscible in all proportions
Reactive Hazards	Substances to be avoided: polymerization initiators, alkali metals, acids, nitrogen oxides, hydrogen peroxide, oxidizing agents, performic acid, organic nitro compounds/bases.
Stability	Tends to polymerize. Unsuitable working materials: various metals.
Health Hazards	After skin contact: Severe irritation. After eye contact: Severe irritation. After ingestion: Irritant effect (mouth, pharynx, oesophagus, gastrointestinal tract). Risk of perforation in the oesophagus and stomach. Inhalation may lead to the formation of oedemas in the respiratory tract. Lacrimal irritation due to vapours. Sensitization with allergic manifestations. Systemic effect: narcosis, blindness.
Toxicity Data	Has been found to cause cancer in laboratory animals. Evidence of reproductive effects. Carcinogen, Category 3.

Formaldehyde hazard data sheet continued

Ecological Data	Do not allow to enter drinking water supplies, waste water, or soil! Caustic even in diluted form.
Disposal	Chemical residues are generally classified as special waste, and as such are covered by regulations which vary according to location. Contact your local waste disposal authority for advice, or pass to a chemical disposal company. Rinse out empty containers thoroughly before returning for recycling.
UN Number	2209
UN Class	9
IATA Number	2209
IATA Packing Group	III
IATA Shipping Name	FORMALDEHYDE SOLUTIONS
IMO Number	9/2209
IMO Packing Group	III
ADR/RID Number	8,63'(c)
Hazard Symbols	T Toxic
Risk Phrases	R23/24/25-34-40-43 Toxic by inhalation, in contact with skin and if swallowed. Causes burns. Possible risk of irreversible effects. May cause sensitization by skin contact.
Safety Phrases	S26-36/37/39-45-51 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves and eye/face protection. In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible). Use only in well ventilated areas
UK Exposure Limits	MEL, Long-term, mg/m3: 2.5 - Formaldehyde

Protocol for paraffin embedding

STEP	REAGENT	TIME	TEMP	AGITATION LEVEL	VACUUM
1	10% NBF	30 mins	Ambient	Stir 5	On
2	100% IMS	45 mins	Ambient	Stir 5	On
3	100% IMS	45 mins	Ambient	Stir 5	On
4	100% IMS	45 mins	Ambient	Stir 5	On
5	100% IMS	45 mins	Ambient	Stir 5	On
6	100% IMS	45 mins	Ambient	Stir 5	On
7	100% IMS	45 mins	Ambient	Stir 5	On
8	Xylene	45 mins	Ambient	Stir 5	On
9	Xylene	45 mins	Ambient	Stir 5	On
10	Xylene	45 mins	Ambient	Stir 5	On
11	Paraffin Wax	1hr30	60°C	Stir 5	On
12	Paraffin Wax	1hr30	60°C	Stir 5	On
13	Paraffin Wax	1hr30	60°C	Stir 5	On

NBF = Neutral Buffered Formalin, IMS = industrial methylated spirit

The samples sit in 10% NBF for between 3-6hrs prior to the protocol starting. During this time the formalin is at ambient temperature (around 30 degrees Celsius), the agitation level is 5 (the highest level) but the vacuum is off.

The processor is an Excelsior ES (Thermo Scientific)
Fisher Scientific Ltd
Bishop Meadow Rd.
Loughborough
LE11 5RG

Appendix B: Data in Excel format

Contents

Microscopy data, Chapter 3

Foal	Region	DOB	Date	Age in days	Area mm²	Epidermis width mm	Largest tubule mm	Number tubules	Density mm²
1	Dorsal	12/2/13	26/6/13	134	2.62	2.62	0.12	103	39.3
1	Lateral	12/2/13	26/6/13	134	2.04	2.04	0.15	68	33.3
1	Medial	12/2/13	26/6/13	134	2.98	2.98	0.14	115	38.5
2	Dorsal	17/4/13	28/6/13	72	4.45	4.45	0.17	145	32.6
2	Lateral	17/4/13	28/6/13	72	3.32	3.32	0.14	133	40.1
2	Medial	17/4/13	28/6/13	72	2.57	2.57	0.12	105	40.8
3	Dorsal	13/3/13	6/6/13	85	3.26	3.26	0.21	79	24.2
3	Lateral	13/3/13	6/6/13	85	2.61	2.61	0.18	101	38.7
3	Medial	13/3/13	6/6/13	85	2.75	2.75	0.15	113	41.1
4	Dorsal	12/2/13	6/4/13	53	5.61	5.61	0.23	206	36.7
4	Lateral	12/2/13	6/4/13	53	2.73	2.73	0.13	127	46.6
4	Medial	12/2/13	6/4/13	53	2.50	2.50	0.16	121	48.3
5	Dorsal	12/2/13	6/4/13	53	4.81	4.81	0.19	170	35.3
5	Lateral	12/2/13	6/4/13	53	2.74	2.74	0.18	139	50.8
5	Medial	12/2/13	6/4/13	53	2.58	2.58	0.16	139	53.9
6	Dorsal	5/3/13	27/6/13	115	2.72	2.72	0.11	85	31.3
6	Lateral	5/3/13	27/6/13	115	3.32	3.32	0.15	106	31.9
6	Medial	5/3/13	27/6/13	115	3.00	3.00	0.12	118	39.3
7	Dorsal	6/5/13	6/5/13	0	2.93	2.93	0.14	122	41.7
7	Lateral	6/5/13	6/5/13	0	2.34	2.34	0.12	106	45.2
7	Medial	6/5/13	6/5/13	0	2.55	2.55	0.12	109	42.8
8	Dorsal	6/5/13	6/5/13	0	2.76	2.76	0.15	91	32.9
8	Lateral	6/5/13	6/5/13	0	2.46	2.46	0.13	106	43.0
8	Medial	6/5/13	6/5/13	0	2.27	2.27	0.11	80	35.3
9	Dorsal	6/3/13	6/3/13	0	3.61	3.61	0.16	114	31.6
9	Lateral	6/3/13	6/3/13	0	2.04	2.04	0.11	86	42.1
9	Medial	6/3/13	6/3/13	0	2.16	2.16	0.09	76	35.3
10	Dorsal	26/2/13	10/4/13	43	3.39	3.39	0.16	123	36.2
10	Lateral	26/2/13	10/4/13	43	2.57	2.57	0.14	121	47.0
10	Medial	26/2/13	10/4/13	43	2.89	2.89	0.11	120	41.6
11	Dorsal	16/3/12	22/1/13	-23	2.57	2.57	0.16	116	45.1
11	Lateral	16/3/12	22/1/13	-23	1.93	1.93	0.09	81	42.0
11	Medial	16/3/12	22/1/13	-23	2.11	2.11	0.13	95	45.0
12	Dorsal	6/3/12	28/12/12	-38	2.82	2.82	0.15	120	42.6
12	Lateral	6/3/12	28/12/12	-38	1.74	1.74	0.11	72	41.5
12	Medial	6/3/12	28/12/12	-38	2.15	2.15	0.11	87	40.6
13	Dorsal	15/3/12	10/1/13	-34	2.17	2.17	0.14	84	38.7
13	Lateral	15/3/12	10/1/13	-34	1.09	1.09	0.09	48	44.2
13	Medial	15/3/12	10/1/13	-34	1.34	1.34	0.11	56	41.9
14	Dorsal	23/4/13	23/4/13	0	3.05	3.05	0.18	103	33.8
14	Lateral	23/4/13	23/4/13	0	2.17	2.17	0.14	93	42.9
14	Medial	23/4/13	23/4/13	0	2.31	2.31	0.14	88	38.0
15	Dorsal	10/3/13	10/3/13	0	2.84	2.84	0.14	122	43.0
15	Lateral	10/3/13	10/3/13	0	2.28	2.28	0.11	99	43.4
15	Medial	10/3/13	10/3/13	0	1.97	1.97	0.10	97	49.3

Radiographic and morphometric data, Chapter 4

Abbreviations and Variables

Age in days

LF; Left front

RF; Right front

DPA; PIII dorsal parietal angle

SPA; PIII solar parietal angle

SBA; PIII solar border angle

PIII W; PIII width (mm)

HWX; Hoof width by radiograph (mm)

PID; Proximal integument depth (mm)

DID; Distal integument depth (mm)

DHWA; Dorsal hoof wall angle

PA; Phalangeal axis

HPA; Hoof pastern axis

width; Width of foot (mm)

length; Hoof length (mm)

hw; Heel width (mm)

Radiographic and morphometric data, Chapter 4, part 1

Foal	Age days	LF DPA	LF SPA	LF SBA	LF PIII W	LF HWX	RF DPA	RF SPA	RF SBA	RF PIII W	RF HWX	LF PID	LF DID	RF PID	RF DID
1	48	52.43	22.59	7.89	52.03	66.17	52.78	21.95	8.12	51.87	66.33	10.18	6.96	10.58	7.29
2	71	63.01	29.22	13.83	53.23	67.20	58.46	22.07	9.30	54.37	70.30	10.20	8.96	9.84	7.72
3	33	49.84	22.15	7.35	54.87	70.37	52.79	21.74	6.69	54.03	69.60	9.98	7.53	10.85	7.17
4	139	57.96	23.95	6.68	57.73	75.43	52.65	21.10	4.19	57.73	75.63	12.49	8.78	12.07	9.46
5	116	50.82	20.07	6.10	60.07	79.73	51.04	18.56	6.36	61.37	81.80	13.04	8.35	14.29	9.65
6	112	53.11	20.81	7.20	60.63	76.30	53.59	22.21	6.80	57.73	73.63	11.29	8.49	12.22	8.60
7	43	49.30	19.80	3.80	51.13	67.17	57.16	24.71	8.08	51.00	67.07	10.18	6.24	10.28	7.13
8	51	48.58	22.44	4.24	54.67	73.67	48.84	19.98	4.24	54.57	73.27	10.91	7.83	11.41	7.67
9	122	54.16	24.68	6.61	59.43	80.67	57.74	26.26	9.89	58.30	78.70	12.11	8.49	12.05	9.97
10	109	51.44	21.87	6.40	57.87	76.50	51.20	20.39	6.13	58.23	75.23	12.15	8.54	12.61	8.30
11	54	52.41	23.12	5.35	54.10	69.97	53.72	22.36	7.13	53.90	68.40	9.87	7.53	10.61	7.88
12	108	55.59	22.87	5.05	55.60	69.50	54.78	20.78	6.55	55.10	69.50	11.50	8.06	12.43	8.38
13	83	52.98	24.08	5.43	53.87	69.07	52.78	22.59	6.91	53.27	69.53	11.67	8.65	11.49	8.11
14	126	48.98	21.71	5.52	60.47	82.10	51.69	26.41	7.15	62.27	83.00	13.60	8.65	11.62	8.97
15	145	54.42	20.16	6.26	60.50	78.73	49.63	18.97	3.62	59.20	75.80	13.30	9.90	12.61	9.16
16	91	54.00	21.93	7.60	58.57	81.97	56.51	26.97	9.04	59.40	79.60	12.46	7.80	12.23	8.14
17	145	53.79	22.63	7.75	58.30	77.27	55.72	21.14	6.94	59.40	77.93	12.26	8.40	12.93	8.47
18	101	47.84	18.85	5.74	57.37	72.83	46.10	17.55	4.53	58.43	73.77	10.14	8.01	10.38	7.89
19	41	52.60	19.76	4.30	52.43	66.50	55.62	23.91	7.79	51.90	65.90	10.14	7.78	10.41	7.87
20	117	52.05	19.44	7.85	58.80	74.00	51.90	18.86	6.91	58.50	74.73	11.35	8.29	11.51	7.94
21	108	53.49	21.58	5.97	62.80	81.27	54.45	22.85	7.22	64.90	82.63	10.88	8.57	11.36	8.27
22	106	52.17	19.77	5.67	55.50	76.87	48.74	18.96	4.19	55.93	77.10	10.44	7.50	11.21	7.71
1	195	50.51	17.68	4.62	63.70	87.07	55.40	23.59	6.56	63.67	87.47	12.45	9.39	13.35	11.54
2	218	53.00	18.02	2.96	63.17	88.83	50.27	18.17	3.09	64.33	91.20	12.83	11.12	12.50	10.16
3	180	50.89	16.62	5.33	63.90	90.23	53.99	21.82	7.53	62.93	87.33	12.53	10.23	12.93	11.93
4	286	58.66	27.92	7.21	65.60	92.33	52.01	19.61	2.08	67.37	94.50	13.73	13.37	12.85	11.88
5	263	46.24	13.19	2.59	65.43	92.73	47.74	15.83	3.48	66.87	93.77	14.55	12.22	15.82	13.99
6	259	45.80	16.97	3.11	64.30	92.07	53.99	20.42	6.04	62.30	86.87	11.99	11.40	12.28	11.76
7	190	49.01	17.22	3.19	61.73	87.13	51.36	19.21	4.90	62.67	89.53	12.44	10.52	13.97	12.60
8	198	46.98	17.11	2.62	61.37	88.10	48.71	18.75	4.25	63.13	89.97	13.07	11.34	13.88	12.04
9	269	52.16	21.46	5.70	63.63	94.43	55.29	26.86	7.72	63.77	93.33	12.88	12.37	13.16	13.00
10	260	48.38	18.38	5.38	68.17	98.90	48.16	17.10	2.46	71.10	97.77	15.03	13.57	14.72	13.81
11	205	52.02	21.02	4.84	66.47	90.20	53.00	21.98	5.94	73.37	92.73	13.48	10.91	14.61	12.79
12	255	50.35	17.21	1.53	64.77	96.63	52.93	18.59	2.43	65.57	91.87	13.98	11.86	14.16	12.42
13	234	54.65	23.66	7.50	66.93	92.13	47.86	18.58	4.28	65.67	89.13	14.98	14.06	12.82	11.21
14	277	46.07	17.55	1.72	69.40	102.13	49.83	19.99	3.45	69.73	103.73	13.62	12.30	15.32	14.54
15	292	51.58	16.71	3.76	71.10	98.23	49.15	17.45	2.40	70.90	101.27	14.87	13.58	13.07	10.92
16	238	53.13	20.84	4.88	67.27	97.47	53.59	22.55	6.40	68.03	98.43	13.42	11.53	13.43	12.15
17	296	54.20	19.21	4.67	67.33	98.73	52.49	16.47	2.85	71.35	101.23	12.86	12.71	13.55	12.62
18	248	48.21	15.91	5.59	66.67	90.83	50.85	17.29	5.89	67.43	91.07	12.44	8.91	13.07	11.04
19	192	54.96	22.44	6.16	63.13	84.37	50.61	18.89	5.84	63.13	83.87	12.62	11.73	11.62	9.98
20	264	50.09	18.33	5.67	65.47	92.33	50.42	17.46	4.24	64.17	89.87	13.46	12.81	12.41	11.68
21	255	49.03	15.53	1.48	67.93	94.17	50.95	19.26	4.69	67.47	92.93	12.85	11.74	13.34	11.27
22	253	47.90	16.88	5.37	63.70	93.87	50.39	16.74	6.06	62.03	89.20	12.20	11.29	12.99	11.77

Radiographic and morphometric data, Chapter 4, part 2

Foal	Age days	LF DHWA	RF DHWA	LF PA	RF PA	LF HPA	RF HPA	LF width	LF length	LF hw	RF width	RF length	RF hw
1	48	58.40	58.17	-3.29	-8.08	5.97	5.38	69	70	47	69	70	43
2	71	65.03	62.71	-2.12	-1.22	2.02	4.25	70	75	44	73	70	49
3	33	54.07	60.37	-3.68	-13.73	4.24	7.58	73	70	46	73	70	42
4	139	66.99	58.92	-2.60	-7.88	9.02	6.27	80	78	55	79	80	56
5	116	59.83	61.80	-4.63	-13.16	9.01	10.76	83	81	50	84	83	61
6	112	56.65	60.15	-3.62	-5.38	3.54	6.56	78	80	55	76	75	50
7	43	56.32	62.86	-3.15	-18.80	7.03	5.69	70	68	44	69	68	44
8	51	54.97	57.12	-3.74	-12.54	6.39	8.28	75	75	51	75	70	51
9	122	63.07	62.65	-2.08	-0.56	8.91	4.91	84	82	59	83	84	57
10	109	57.08	59.14	-4.32	-11.44	5.64	7.94	80	80	54	79	80	51
11	54	57.52	56.66	-2.73	-10.20	5.11	2.93	72	65	45	70	68	45
12	108	59.35	61.81	-4.05	-13.42	3.76	7.03	74	80	45	85	80	45
13	83	62.32	60.43	-3.38	5.44	9.34	7.65	71	73	45	71	69	50
14	126	55.27	58.32	-2.65	-15.08	6.29	6.63	85	90	53	87	91	55
15	145	61.78	57.35	-3.45	-19.16	7.36	7.72	85	85	53	83	80	53
16	91	58.12	64.74	-4.09	-11.54	4.12	8.23	85	80	60	83	82	57
17	145	61.62	63.19	-4.46	-0.67	7.83	7.46	83	85	55	85	84	57
18	101	50.97	49.37	-2.50	-19.56	3.14	3.27	76	78	42	76	77	42
19	41	55.93	61.35	-2.54	-12.19	3.33	5.72	68	69	37	69	69	40
20	117	56.92	58.14	-3.56	-16.89	4.87	6.24	78	78	51	78	78	51
21	108	58.56	62.71	-3.09	-16.11	5.07	8.26	85	85	55	84	79	52
22	106	54.39	54.44	-3.50	-22.49	2.23	5.70	78	76	51	78	80	52
1	195	55.21	58.62	-0.75	-4.28	4.70	3.21	113.5	111	66	110	101	66
2	218	55.11	53.43	-8.37	-13.52	2.11	3.17	98.5	94	62	97.5	92	63
3	180	53.86	54.92	-11.58	-10.68	2.98	0.93	97	91	60	112	93.5	58
4	286	60.05	54.11	-7.76	-12.19	1.39	2.10	110	116	68	114	109	67
5	263	51.13	49.95	-13.12	-12.39	4.89	2.21	91	90	51	91	88	55
6	259	46.33	55.70	-14.96	-5.68	0.53	1.71	113.5	101	65	112	111	64.5
7	190	52.59	53.74	-7.03	-7.35	3.58	2.38	94.5	93	58	96	91	59
8	198	49.90	52.61	-10.31	-9.04	2.92	3.90	94.5	90	60.5	93.5	59	49
9	269	51.36	54.87	-8.79	-7.36	-0.80	-0.42	96	92.5	61	92.5	90	50
10	260	51.76	49.93	-12.16	-6.88	3.37	1.77	93	90	61	93.5	90	52
11	205	55.61	55.60	-6.47	-1.80	3.59	2.60	101.5	99	71	102.5	96	63.5
12	255	53.24	56.63	-7.89	-4.99	2.89	3.70	110	101	61	103	94.5	68
13	234	56.39	49.33	-4.85	2.73	1.74	1.47	103	100	65	101.5	98	60
14	277	49.53	52.28	-14.38	-10.27	3.46	2.45	100	92.5	59	94.5	92.5	52.5
15	292	54.98	54.17	-15.25	-4.20	3.40	5.03	96.5	95	55	95.5	92	52
16	238	54.08	55.77	-2.25	-6.45	0.95	2.18	103	96	61	101	96.5	55
17	296	55.35	53.31	-3.15	-2.42	1.15	0.82	105	100	61.5	105.5	96.5	64
18	248	54.26	55.77	-10.04	-8.86	6.05	4.91	98.5	95	66.5	95	89	55
19	192	57.87	53.65	-9.17	-9.45	2.91	3.04	106	110	56	105	98	63
20	264	51.08	50.56	-15.79	-9.26	0.99	0.14	104	98	64	98	98	56
21	255	53.94	54.26	-14.69	-0.44	4.91	3.31	93.5	90	50	97	93	50
22	253	48.47	51.10	-7.65	-13.90	0.57	0.70	95	91	56	97	89	53

Hoof growth and compression data, Chapter 5

Abbreviations and Variables

Age in days

Days measured

HGM; Hoof growth measurement (mm)

Comp. D; Compression distance (mm)

Toe length (mm)

Comp. Res. %; Compression residual %

Hoof Growth (mm)

HGR;Hoof growth rate (mm/day)

Nd; No data

Weanling	Age days	Days measured	HGM (mm)	Comp. D (mm)	Toe length (mm)	Comp. Res. %	Hoof Growth (mm)	HGR (mm/day)
1	324	0	21.7	15.2	71.8	100.00	0.00	0.00
1	352	28	28.0	14.8	69.9	97.46	6.31	0.23
1	378	54	33.6	14.5	73.1	95.29	11.95	0.22
1	406	82	44.8	14.1	75.5	92.85	23.14	0.28
1	429	105	49.3	13.6	74.6	89.58	27.62	0.26
1	456	132	57.3	13.3	74.8	87.50	35.64	0.27
1	484	160	67.1	nd	75.3	nd	45.36	0.28
2	253	0	20.1	14.8	65.7	100.00	0.00	0.00
2	282	29	25.3	14.7	67.1	99.28	5.22	0.18
2	309	56	30.6	14.7	65.9	99.30	10.48	0.19
2	382	129	44.6	13.9	71.7	93.77	24.45	0.19
2	394	141	55.8	nd	72.1	nd	35.66	0.25
3	266	0	19.3	14.5	62.6	100.00	0.00	0.00
3	295	29	28.3	14.5	68.2	99.73	8.97	0.31
3	322	56	31.5	14.0	68.7	96.43	12.21	0.22
3	364	98	45.4	14.0	72.0	95.95	26.05	0.27
3	407	141	55.7	13.2	73.6	90.67	36.36	0.26
3	450	184	69.3	nd	73.8	nd	49.99	0.27
4	269	0	25.2	14.6	71.2	100.00	0.03	0.00
4	316	47	33.0	14.0	74.3	95.69	7.83	0.17
4	353	84	39.1	13.8	75.7	94.39	13.94	0.17
4	409	140	64.3	13.2	83.9	90.21	39.12	0.28
4	430	161	71.7	nd	85.0	nd	46.52	0.29
5	292	0	24.2	15.2	70.4	100.00	0.00	0.00
5	320	28	29.4	14.8	69.2	97.25	5.27	0.19
5	346	54	39.4	14.2	72.3	93.91	15.20	0.28
5	374	82	41.5	14.2	75.9	93.89	17.39	0.21
5	424	132	54.4	14.2	75.6	93.69	30.22	0.23
5	453	161	64.4	nd	74.1	nd	40.28	0.25
6	260	0	24.2	14.7	70.9	100.00	0.00	0.00
6	289	29	31.1	13.8	74.0	93.97	6.93	0.24
6	316	56	41.0	13.5	74.5	91.70	16.80	0.30
6	358	98	47.7	13.2	77.0	89.95	23.53	0.24
6	401	141	68.5	nd	76.6	nd	44.34	0.31
7	307	0	31.5	13.9	77.1	100.00	0.00	0.00
7	333	26	41.8	13.7	76.5	98.75	10.32	0.40
7	361	54	47.2	13.7	76.5	98.46	15.76	0.29
7	384	77	53.7	13.4	78.0	96.66	22.19	0.29
7	411	104	62.4	13.3	78.0	95.82	30.91	0.30
7	440	133	72.3	nd	81.6	nd	40.82	0.31
8	287	0	27.9	16.0	74.2	100.00	0.00	0.00
8	314	27	30.3	15.4	76.5	95.91	2.43	0.09
8	356	69	39.5	15.2	78.2	94.68	11.67	0.17
8	399	112	58.1	14.9	81.6	92.83	30.20	0.27
9	318	0	22.9	15.5	65.2	100.00	0.00	0.00
9	347	29	29.2	15.0	67.9	96.54	6.33	0.22
9	374	56	36.1	14.6	68.1	94.23	13.18	0.24
9	416	98	45.0	14.3	70.4	92.17	22.16	0.23
9	459	141	59.2	nd	71.6	nd	36.30	0.26
10	261	0	16.6	19.7	61.6	100.00	0.00	0.00
10	317	56	28.5	19.6	71.2	99.63	11.90	0.21
10	359	98	38.6	19.5	73.7	98.98	22.01	0.22
10	402	141	55.2	19.1	77.5	97.39	38.67	0.27
11	270	0	23.1	14.7	66.9	100.00	0.00	0.00
11	326	56	32.3	14.5	74.5	98.50	9.24	0.17
11	368	98	42.6	13.9	75.2	94.37	19.57	0.20
11	411	141	55.6	13.4	77.6	91.10	32.50	0.23
11	454	184	71.8	nd	79.9	nd	48.71	0.26
12	317	0	23.1	13.6	73.0	100.00	0.00	0.00
12	345	28	36.1	13.3	76.5	98.18	12.99	0.46
12	371	54	40.8	13.2	78.5	97.06	17.75	0.33
12	399	82	41.5	12.9	73.3	95.17	18.46	0.23
12	422	105	51.5	12.8	78.0	93.89	28.43	0.27
12	449	132	57.9	12.4	80.1	91.56	34.85	0.26
12	478	161	60.8	12.2	79.1	89.70	37.77	0.23
12	496	179	72.2	nd	81.0	nd	49.13	0.27

Hoof growth, hoof compression, and hoof loading data, Chapter 6

Abbreviations and variables

LFM HGR = left fore medial hoof growth rate (mm/day);

LFD HGR = left fore dorsal hoof growth rate (mm/day);

LFL HGR = left fore lateral hoof growth rate (mm/day);

RFM HGR = right fore medial hoof growth rate (mm/day);

RFD HGR = right fore dorsal hoof growth rate (mm/day);

RFL HGR = right fore lateral hoof growth rate (mm/day);

LFM Comp = left fore medial compression rate (mm/day);

LFD Comp = left fore dorsal compression rate (mm/day)

LFL Comp = left fore lateral compression rate (mm/day);

RFM Comp = right fore medial compression rate (mm/day)

RFD Comp = right fore dorsal compression rate (mm/day);

RFL Comp = right fore lateral compression rate (mm/day)

LF DHWA = left fore dorsal hoof wall angle;

RF DHWA = right fore dorsal hoof wall angle

LFM load = left fore medial percentage loading;

LFL load = left fore lateral percentage loading

LFD load = left fore dorsal percentage loading;

LFC load = left fore caudal percentage loading

RFM load = right fore medial percentage loading;

RFL load = right fore lateral percentage loading

RFD load = right fore dorsal percentage loading;

RFC load = right fore caudal percentage loading

Hoof growth, hoof compression, and hoof loading data, part 1

Foal	Group	LFM HGR	LFD HGR	LFL HGR	RFM HGR	RFD HGR	RFL HGR	LFM Comp	LFD Comp	LFL Comp	RFM Comp	RFD Comp	RFL Comp
1	Control	0.41	0.33	0.41	0.43	0.36	0.42	0.03	0.07	0.02	0.14	0.01	0.12
2	Control	0.50	0.51	0.54	0.50	0.49	0.55	0.09	0.04	-0.01	0.07	0.08	0.10
3	Control	0.42	0.38	0.42	0.39	0.38	0.43	0.05	0.04	0.05	0.08	0.05	0.08
4	Control	0.40	0.38	0.43	0.38	0.40	0.43	0.05	0.10	0.03	0.03	0.05	0.02
5	Control	0.42	0.40	0.45	0.37	0.36	0.46	0.07	0.04	0.01	0.03	0.05	0.02
6	Control	0.25	0.27	0.29	0.25	0.26	0.28	0.07	0.06	0.02	0.06	0.06	0.05
7	Control	0.33	0.33	0.38	0.35	0.32	0.33	0.06	0.07	0.05	0.11	0.06	0.08
8	Control	0.45	0.54	0.54	0.44	0.51	0.45	0.04	0.02	0.04	0.03	0.03	0.02
9	Control	0.43	0.44	0.44	0.46	0.41	0.47	0.06	0.04	0.03	0.03	0.04	0.05
10	Control	0.36	0.38	0.41	0.39	0.41	0.43	0.03	0.03	0.03	0.03	0.05	0.02
11	Control	0.52	0.45	0.57	0.45	0.51	0.53	0.02	0.03	0.02	0.04	0.03	0.01
12	Control	0.54	0.46	0.59	0.47	0.52	0.55	0.08	0.05	0.03	0.04	0.05	0.03
13	Control	0.34	0.37	0.44	0.34	0.38	0.39	-0.01	0.02	0.03	0.01	0.01	0.03
14	Control	0.27	0.26	0.29	0.26	0.23	0.28	0.13	0.18	0.13	0.12	0.15	0.12
15	Control	0.29	0.21	0.35	0.19	0.15	0.22	0.04	0.04	0.02	0.04	0.04	0.03
16	Control	0.33	0.34	0.37	0.33	0.34	0.38	0.02	0.02	0.00	0.02	0.02	0.02
17	Control	0.47	0.53	0.61	0.61	0.59	0.67	0.14	0.15	0.05	0.12	0.15	0.17
18	Control	0.43	0.45	0.53	0.47	0.52	0.54	0.04	0.03	0.02	0.04	0.03	0.04
19	AFD	0.41	0.28	0.53	0.47	0.34	0.52	0.03	0.00	0.02	0.10	0.00	-0.01
20	AFD	0.49	0.38	0.40	0.42	0.38	0.48	0.04	0.02	0.03	0.02	0.02	0.01
21	AFD	0.45	0.38	0.46	0.47	0.38	0.42	0.03	0.02	0.02	0.03	0.03	0.02
22	AFD	0.45	0.35	0.49	0.49	0.37	0.50	0.04	0.03	0.02	0.01	0.05	0.02
23	AFD	0.42	0.38	0.40	0.39	0.35	0.44	0.03	0.02	0.01	0.03	0.01	0.01
24	AFD	0.49	0.42	0.53	0.46	0.39	0.49	0.04	0.02	0.02	0.02	0.02	0.03
25	AFD	0.39	0.26	0.39	0.54	0.40	0.41	0.08	0.00	0.01	0.13	0.02	0.07
26	AFD	0.31	0.22	0.25	0.27	0.30	0.31	0.02	0.03	0.02	0.02	0.04	0.07
27	AFD	0.55	0.41	0.55	0.51	0.41	0.64	0.02	0.02	0.02	0.02	0.01	0.04

Hoof growth, hoof compression, and hoof loading data, part 2

LF DHWA	RF DHWA	LFM load	LFL load	LFD load	LFC load	RFM load	RFL load	RFD load	RFC load
58.5	58.7	70	31	25	75	71	29	44	56
54.7	55.6	69	31	58	42	62	38	57	43
56.1	57.8	55	46	52	48	64	36	64	36
51.3	58.3	62	38	62	38	70	30	78	22
53.3	59.3	58	42	40	60	62	38	62	38
51.1	54.5	66	37	49	51	71	29	59	41
60.0	59.8	63	37	40	60	75	25	72	28
60.0	59.9	73	27	40	60	76	24	67	33
54.7	56.5	58	42	53	47	59	41	57	43
57.5	56.9	61	39	49	51	60	40	75	25
55.9	56.5	53	47	43	57	56	44	65	35
61.1	61.2	60	41	35	65	78	22	59	41
57.6	57.7	60	40	44	56	60	40	57	43
60.0	59.6	54	46	45	55	63	37	54	46
64.6	61.3	58	42	66	34	58	42	73	27
58.0	59.7	64	36	36	64	84	16	58	42
59.7	60.8	74	28	51	49	68	32	66	34
57.9	59.7	59	41	46	54	68	32	62	38
59.4	59.9	59	41	68	32	52	48	74	26
60.6	65.9	54	46	65	35	58	42	71	29
60.4	59.1	63	37	70	30	54	46	74	26
60.7	62.1	57	43	70	30	43	57	81	19
59.8	59.7	61	39	76	24	55	45	91	9
55.7	55.4	66	34	60	40	78	22	82	18
58.3	58.6	58	42	55	45	61	39	60	40
58.0	61.1	51	49	72	28	61	39	82	18
63.0	62.0	51	49	73	27	50	50	79	21

Appendix C: Publications during the PhD project

Contents

EXTERNAL AND RADIOGRAPHIC HOOF ANGLES DIFFER IN THOROUGHBRED FOALS

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Introduction: Poor hoof angle and hoof pastern alignment has been linked to lameness in mature horses. External characteristics, such as the hoof pastern axis and hoof angle, are commonly used to assess dorsopalmar conformation in the same manner in foals. The hoof wall integument and distal phalanx is parallel in healthy mature horses but not in foals: this is not understood.

Methods: The front feet of 22 Thoroughbred foals were radiographed and measured in June and again in November. Lateromedial radiographic images were measured (Osirix) for hoof integument angle and width, and proximal, middle and distal phalanx angles. The data were tabulated in Excel and analysed in Minitab. After assessment for normality (Anderson-Darling), potential relationships were assessed for differences between June and November using paired t-tests and regression analysis.

Results: The dorsal hoof angle became more acute with age, $R^2 = 0.3326$, the mean of June, 59.05±3.662°, was significantly different from November, 53.64±2.7824°, $P<0.0001$. The hoof pastern axes were broken forward in June, 6.1±2.1°, in November 2.5±1.6°, whereas the phalangeal axes were broken back, June,-11.0±7.5°, November, -8.3±4.4°. There was a significant relationship between age and hoof pastern axis alignment, $R^2 = 0.3746$; $P<0.0001$. The phalangeal axis did not correlate with age. The relationship between age and parallelism of the integument was significant $R^2 = 0.4867$; $P<0.0001$.

Conclusions: The cause of the non-alignment of the hoof pastern axis and phalangeal axis in Thoroughbred foals is widening of hoof integument proximodistally and circumferential bone thickening of the distal phalanx. Assumptions of phalangeal angles based on external characteristics cannot be made in foals.

Declarations:

1. Approval was given by the University of Central Lancashire Animal Projects Committee.
2. There were no competing Interests.
3. World Horse Welfare partly funded this study.

88

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Short Communication

Hoof renewal time from birth of Thoroughbred foals

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ABSTRACT

A circumferential ring in the hoof horn of foals occurs at birth and grows down to the distal border as the fetal hoof is replaced. Horn growth and complete hoof capsule renewal have not been measured in Thoroughbred foals but the determination of time of hoof renewal may allow accurate predictions of healing time to be made in cases of hoof lesions. The objective of this study was to measure the time taken for the fetal hoof of newborn foals to grow to the distal border and be replaced by hoof grown since birth. The age of the foal in days on the day that routine hoof trimming removed the hoof ring of the front hooves was recorded. The mean age at which the fetal hoof was removed was 145 ± 15 days (95% CI, 141.8–147.2), range 120–165 days. Thoroughbred foals replaced the fetal hoof in approximately half the time taken for mature horses (270–365 days).

Thoroughbred foals stand within minutes of birth on hooves developed in utero which are covered by eponychium, also known as deciduous hoof. This is shed within a few days in a healthy foal (Bragulla, 2003). From birth, a growth ring is present in the hoof wall parallel to the coronary band, and the horn proximal and distal to this line often differs in colour (Ellis, 1998). Smallwood et al. (1989) noted that in lateromedial radiographs of young foals an indent in the hoof wall, described in this paper as a 'foal hoof crease' (FHC), was seen on all four hooves, marking the event of foaling (Fig. 1). However, Butler and Hintz (1977) studied the rate of hoof growth in 14 Shetland pony foals aged 8–11 months and did not mention the FHC.

Hoof wall renewal in all ages of horses has been poorly reported. Kainer (1987) stated that the time for the hoof at the toe to grow to the distal border was 270–365 days. Hoof growth rates in mature horses have been reported without stating hoof wall renewal time (Reilly et al., 1998; Faramarzi et al., 2009). A number of authors have speculated that the hoof wall grows faster in young horses but only two groups measured hoof growth rates in foals (Butler and Hintz, 1977; Smallwood et al., 1989). Neither study investigated the time required from birth to replace the hoof capsule or wall.

Horses often suffer partial hoof wall avulsion that may cause lameness and threaten sale value (Parks, 2008). When there are lesions to the hoof wall it is useful to have an estimation of renewal time. The objective of the current study was to measure the time taken for the fetal hoof of newborn foals to grow to the distal border and be replaced by hoof grown since birth.

Ethical approval was given by the University of Central Lancashire Animal Projects Committee. Thoroughbred foals from four stud farms (n = 150) were assessed prior to and after routine hoof trimming at 3 week intervals. Whether the fetal hoof was visible prior to hoof trimming or not was noted for each foal. All hooves were trimmed by the same experienced farrier. Following foot trimming the presence or absence of FHC was determined in both front hooves. Where the fetal hoof was no longer visible post trim the foal joined the cohort and the age of the foal was recorded. The data were tabulated (Microsft Excel), analysed (Minitab) and assessed for normality using the Anderson–Darling test. Only data from foals with no history of lameness, illness or stable confinement were analysed.

Forty-five foals fulfilled the study criteria. The mean age at which the fetal hoof was removed by trimming was 145 ± 15 days (95% confidence interval, 141.8–147.2), range 120–165 days (Fig. 2.). The Thoroughbred foals renewed their fetal hoof wall at twice the rate estimated in mature horses (Kainer, 1987). This is not surprising as the foot of a foal is smaller and therefore there is a shorter distance for the hoof to grow from the site of origin at the coronary band to the distal border. Additionally, it has been reported that the foal hoof grows faster than that of mature horses (Butler and Hintz, 1977).

The term 'foal foot' has been used to describe hoof distal to the FHC (Ellis, 1998). This is a misnomer as the the hoof distal to the FHC grows in utero and should therefore more correctly be termed 'fetal hoof'. The hoof proximal to the FHC, which grows post partum,

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Fig. 1. A foal, approximately 2 months old, showing the foal hoof crease (arrow), marking the time of birth.

should be termed the 'foal hoof'. The cause of the FHC is not known but may be a consequence of the foal changing from in utero non weight-bearing to weight-bearing following birth. Another factor that may cause the FHC is the change in diet of the foal associated

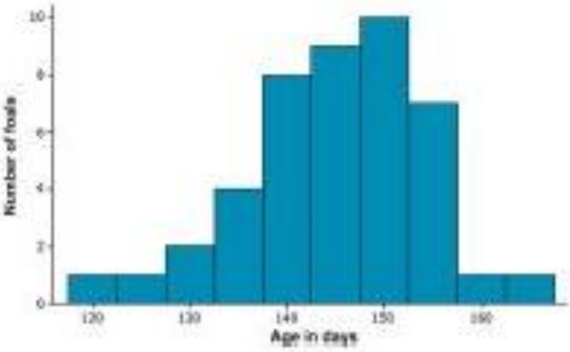

Fig. 2. Histogram of the age at which the foal hoof crease (FHC) was removed by trimming.

with switching from nutients passed via the placenta to milk supplied by the mare and other feed such as grass taken orally (Huntingdon and Pollitt, 2005). In mature horses prominent growth rings have been associated with lameness and sudden changes in dorsal wall angulation (Dyson et al., 2011).

Knowing the time for hoof renewal may allow farriers, horse owners and veterinary surgeons to make an accurate prediction of healing time. In cases of partial hoof wall avulsion in horses, once the initial lesion has been treated and epithelialisation has begun it is useful to be able to predict the time that it will take for the new hoof generated from the coronary band to grow down to the distal border. Submural abscessation often erupts at the coronary band causing a horizontal crack in the hoof wall. The hoof wall will usually break when this crack nears the distal border, and again it is useful to be able to calculate when this may occur in order for preventive strategies to be employed.

The limitations of this study resided in the methodology. As the trimming schedule was every 21 days this may mean that the accuracy of the data was ±10.5 days. Further studies of hoof growth rates in foals and older horses would be beneficial to make similar calculations.

Conflict of interest statement

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper.

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THE HOOF RENEWAL TIME OF THOROUGHBRED FOALS FROM BIRTH

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Introduction: Thoroughbred foals are born with fully developed hooves and stand within minutes. A ring in the horn occurs at birth and grows down the hoof wall, marking the fetal hoof (distal) and foal hoof (proximal). Birth rings in hoof horn have been noted in other species and used to estimate age. Hoof wall renewal in all ages of horse has been poorly reported; horn growth and complete hoof capsule renewal has not been measured in Thoroughbred foals. Partial hoof wall avulsions are relatively common. The objective was to measure the time taken for the fetal hoof of a newborn foal to grow out and be replaced by hoof grown since birth.

Methods: Thoroughbred foals (N=150) on four Newmarket stud farms were studied during June to September. The age of the foal and the day that routine hoof trimming removed the remnants of the fetal hoof of the front hooves was recorded. Foals that were on restricted exercise, ill or where the remnants of the fetal hoof still remained after trimming were excluded from the data. 45 foals fulfilled the criteria. The data were tabulated in Excel, analysed in Minitab and assessed for normality (Anderson-Darling).

Results: The mean age at which the fetal hoof grew out was 145±15 days (95% confidence interval, 141.77-147.16).

Conclusions: Thoroughbred foals replace the fetal hoof at twice the speed given for mature horses (270-365 days). Knowing the time of hoof renewal will allow accurate predictions of healing to be made in cases of partial hoof wall avulsion and other hoof lesions.

Declarations:

1. Approval was given by the University of Central Lancashire Animal Projects Committee.
2. There were no sources of funding
3. No competing Interests.

Foot and Farriery

Chaired by Chris Pardoe

11.00–11.20

Podiatry for flexural deformity in foals and youngstock

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Introduction

Two common types of flexural deformity (FD) affecting the leg of the Thoroughbred (TB) foal are hypoflexion tendons and acquired flexor deformity (AFD) of the distal interphalangeal joint (DIPJ). Both can occur at or before the foal reaches 100 days old. The causes, especially of AFD affecting the DIPJ, are still disputed. This paper describes treatments for both these types of FD.

Hypoflexion tendons

Foals are frequently born with a toe-up conformation, especially in the hind feet. The inverted cone shape of a neonate [1] does not lend itself to caudal support. Weight is therefore borne in the palmar third of the foot, crushing heels so that they under-run forward, often causing lesions to the heel bulbs. Mild cases mostly improve with exercise, but where this condition is intractable it should be treated. In mild but persistent cases trimming the heels back reduces the anterior fulcrum effect. Where this does not produce immediate improvement applying caudal extensions is effective [2].

Treatment with aluminium caudal extension shoes

Aluminium plate of 6 mm thickness is cut to a triangle approximately 150 x 150 x 75 mm and a toe-clip of 15 mm height turned at the apex. The hooves are trimmed to normal proportions, all hoof wall, sole and frog cleaned by trimming and the shoe attached using methylmethacrylate (acrylic) glue (Jameg glue)[a] reinforced by 3 bands of glue-impregnated fibreglass cloth cut 20 x 100 mm. The foal must be exercised and the extensions removed between 2 and 3 weeks [3]. Where improvement is not satisfactory, the treatment should be repeated.

Flexor tendon deformity of distal interphalangeal joint

Acquired flexor deformity of the DIPJ has 2 phases; initially a heel-up stance, usually in both front feet; this condition may progress to a second phase (club foot). In Thoroughbreds the heel-up stance occurs at 20–110 days old and untreated may cause unilateral club foot in less than 2 months [4]. Suggested causes have included nutrition, rapid skeletal growth, and pain [5]. None of these has been established through repeatable studies and although pain as a cause is the current theory in Newmarket, it appears illogical and was dismissed many years ago [6]. As age is a highly significant factor, this should be a focus for study.

Club foot has been described as a dorsal hoof wall angle (DHWA) of greater than 60° [7], as 30- to 120-day-old TB foals were found to have a mean >59° this is a misleading definition (unpublished data). A club foot is more than the angle of the dorsum and usually includes high heels, concave toe and distension of the white line at the toe [8].

Treatment with aluminium toe extension

The same procedure is used whether the foal is in phase 1 or 2 of the condition. The hoof is trimmed to a normal hoof angle and proportion; all hoof, including wall, sole, and frog is finely trimmed to clean horn. Aluminium plate (2–3 mm thick) is cut 15 mm wide and to required length. The aluminium is bent so that it begins at the anterior frog, projects 15–20 mm anterior to the toe and back to the dorsum, approximately 15 mm below the coronary band. Polymer urethane glue[b] is applied to the sole, frog and dorsum and the aluminium embedded into it. More glue is applied over the aluminium to a depth of approximately 3 mm. The foot is drawn forward and more glue applied across the aluminium to attach it to the hoof wall. Care is taken not to get glue on the coronary band or hairline [3].

The foal cannot be exercised once the toe extension is applied and is kept on box rest or in a small barn (approximately 300 square metres). The toe extension is removed after 3 weeks and the hoof trimmed, the foal is inspected on a firm level surface to decide whether to repeat the treatment.

The first author has used the above technique for many years. Most cases show improvement and although success is subjective, phase 1, heels-up cases usually maintain normal foot conformation on follow-up. Phase 2 (club foot *grades 1* and *2*) cases also usually show improvement, although some remain uneven footed, that is with the affected DHWA greater than that of the opposing hoof.

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Manufacturers' addresses

[a]Jameg Glue, Barnby Moor Stables, Kennel Drive, Barnby Moor, Retford, UK.
[b]Adhere, Vettec: Newmarket Farrier Supply, Moulton Rd., Newmarket, UK.

12th Geneva Congress on Equine Medicine and Surgery (Organization: Pierre Chuit, Founex, Dr Stephane Montavon, Pont-la-Ville, Cristina Pococco-Zindel, Vulbens), Geneva (CH), 11-13 December 2012

THE INCIDENCE OF ACQUIRED FLEXURAL DEFORMITY AND UNILATERAL CLUB FOOT (UNEVEN FEET) IN THOROUGHBRED FOALS

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Aims: *To measure the incidence of acquired flexural deformity (AFD) affecting the distal interphalangeal joint, causing heel-up conformation (AFDdipj) and unilateral club foot (UCF) in a sample population of Thoroughbred foals.*
Methods: *An observational study of Thoroughbred foals (n=373) between 2006 and 2009.*
Results: *The incidence of AFDdipj by age was very significant (Anderson-Darling, s.d. 22.75, P< 0.01) mean 53.5 days, range 20 to 110 days. AFDdipj to UCF was a highly significant association (P< 0.001 by χ^2). There was a highly significant association of laterality (P< 0.001 by χ^2).*
Conclusions: *The incidence by age is earlier than previous literature has stated and therefore the causes of AFD affecting the DIP joint may need to be reassessed. This study has given useful first time data on these conditions in Thoroughbred foals in the UK, which has challenged previous wisdom.*

INTRODUCTION

This is a retrospective study of a sample population (n=373) of Thoroughbred (TB) foals to establish the incidence of acquired flexural deformity (AFD), affecting the distal interphalangeal joint (DIPJ) and club foot (CF) from 2006-2009. CF in foals and later in adults, may impede performance, increase injuries and reduce monetary value. The incidence and timing of the flexural deformity syndrome have not been well defined and there is little literature on this subject, greater understanding may lead to more rational intervention treatment.

Fig. 1: Ballerina Syndrome, where the heels remain off the ground even at the walk; note that hoof shape is still normal.

AFD represents a deviation of the leg in the sagittal plane which is related to the persistent hyperflexion of a joint or joints including distal interphalangeal, metacarpo-interphalangeal and carpal joints [1]. This study uses the term; acquired flexural deformity affecting the distal interphalangeal joint (AFDdipj) to describe when a foal is unable to fully load the heel to bear weight. This is commonly called "heels-up," or in severe cases "ballerina syndrome" [2] *(Figure 1)*.

Club foot, when affecting one front foot, is described as unilateral club foot (UCF). A CF is a hoof capsule with a number of visually recognisable distortions from the norm. A grade 1 CF is one where the dorsal hoof wall angle (DHWA) is 3-5° steeper than the opposing foot and a grade 2 CF is where the DHWA angle is 5-8° greater than the opposing foot [3] *(Figure 2).*

The foals age at which the general condition of AFD occurs, is given as between 6 weeks to 8 months [4], 6 weeks to 6 months [5], 1 week to 6 months [6], 1 to 4 months [7], and 4 to 12 months [8]. There is inconsistency between these authors and no data given to substantiate claims about age of occurrence, which range between one week and 12 months, and time of year of AFD occurrence.

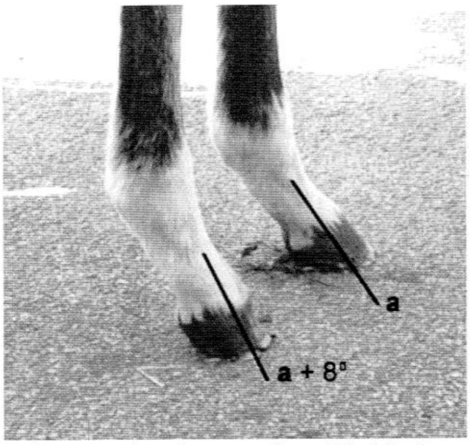

Fig. 2: *a, normal angle compared to a+8°, defined as a grade 2 club foot.*

Untreated AFDdipj leads to changes to the hoof capsule resulting in CF [9]. Distortions described include increased wear at the toe due to the heel-up conformation causing excessive weight bearing to this area and/or increased heel growth due to non-weight-bearing [10]. There seems to be no data confirming the belief that CF could be the result of an untreated or unsuccessfully treated AFDdipj. A survey of TB foals in Japan recorded the incidence of CF but did not report on AFDdipj [11].

Laterality, the preference of using one limb rather than the opposing limb, has been shown to occur in horses [12] and it has been proposed that laterality may lead to uneven feet [13].

The aims of this study were:

1. To measure the incidence of AFDdipj in the sample population (n=373),
2. To measure the incidence of UCF,
3. To examine whether the age of the foal is a factor in AFD,
4. To examine any association of incidence between AFD and time of year,
5. To examine any association between AFDdipj and UCF,
6. To examine whether laterality is a factor in UCF,
7. To examine whether the time of year foaled is a factor in AFD.

MATERIALS AND METHODS

The stud boarded a permanent herd of approximately 30 broodmares and additional seasonal mares which stayed at the farm to foal and to be mated with local stallions before leaving. The stud also prepared foals for sale and therefore the number of foals at the stud continually changed.

Methods:

During their time at stud, each foal was assessed by the leading author every three to four weeks. At the assessment, each foal was led from its stable accompanied by its dam. The foal was walked and assessed from a lateral view-point. The foal was stood in a balanced pose with all four limbs bearing weight. The foal was made to shift its weight to ensure a relaxed posture and stand with the fore-legs close to parallel and not splayed. The lead author viewed the legs and hooves while squatting approximately 3m away. The dorsal hoof wall angle was assessed in comparison with the opposing hoof. Ground contact was determined visually and where the heels appeared not to bear weight the foal was moved in an attempt to make it place its heels firmly on the ground.

Any reference to previous notes on the foal was delayed until after the assessment was recorded in the notebook so that each foal was viewed as if it was the first time seen. Each foal was denoted by its dam's name and year of birth and the date of the assessment were recorded. Conformational variances from ideal were noted by leg and the grading of the disparity in DHWA was by the Redden method [13]. Conformation was also evaluated as "flexor tendons tight" where weight-bearing did not leave the foals heels firmly on the ground and "heels-up" where there was a clear gap between the ground and the heels of at least 5mm (Figure 1). During the compilation of data, flexor tendons tight, heel-up and ballerina syndrome were combined as AFDdipj and CF grades 1 and 2 were combined as unilateral club foot (UCF) *(Figure 2).* AFDdipj and UCF were also recorded as a combined figure of AFD.

Statistical analysis :

All data were entered into Excel[1] and transferred for analysis by Minitab[2]. Foals were annotated by their dam's name and year of birth and also their date of birth. Each assessment was entered as a new line with categories of date, right fore and left fore (RF and LF) with foot and leg conformation entered as; no comment (nc), AFDdipj or UCF. For analysis, cases of AFDdipj and UCF were only entered on their first recorded date.

An Anderson-Darling test for normality was used to test :
1. incidence of AFDdipj by age and day of the year;
2. incidence of UCF by age and day of the year.

Pearson chi-square (χ^2) analysis was used to test for associations between :
1. AFDdipj and UCF;
2. the prevalence of laterality in UCF,
3. the month born and AFDdipj,
4. month born and UCF. Significance was set at $P \leq 0.05$.

Results:

Incidence of AFD in the population :

There were 116 cases of AFDdipj and UCF and 257 foals which did not present with either AFDdipj or UCF and were referred to as "healthy" foals. As 23 of the foals were noted as AFDdipj and UCF at some point, then the figure is arrived at by $\sum$= (AFDdipj + UCF) – 23 = 67 + 72 – 23 = 116 AFD cases). The percentage of foals noted as either AFDdipj or UCF during the year was 31% of the population *(Table 1).*

The 67 foals assessed as AFDdipj were recorded at 53.5 days of age (mean) and 54 days of age (median). The range for AFDdipj was 20 days - 110 days. The incidence of AFDdipj by age was very significant (Anderson-Darling, s.d. = 22.75, P < 0.01) *(Figure 3).* No foal was seen with a UCF before 53 days of age and the oldest that a foal was when first assessed with a UCF was 242 days. On checking the data, this was the first time that this foal was presented to the leading author and was therefore considered an anomaly. The incidence of UCF by age was not significant (Anderson-Darling, s.d. = 39.82, P = 0.092) *(Figure 4).*

Table 1: *Summary of AFDdipj and UCF incidence*

Year	Foals	Healthy (%)	AFD (%)	AFDdipj (%)	UCF (%)	AFDdipj / UCF (%)
2006	89	65 (73)	24 (27)	12 (14)	18 (21)	6 (50)
2007	106	76 (72)	30 (29)	22 (20)	12 (12)	4 (18)
2008	99	68 (69)	31 (31)	15 (16)	23 (24)	7 (47)
2009	79	48 (61)	31 (39)	18 (23)	19 (24)	6 (34)
Totals	373	257 (69)	116 (31)	67 (18)	72 (19)	23 (35)

AFD = acquired flexural deformity; Healthy = foals not seen with AFDdipj or UCF; AFDdipj = foal with heel-up conformation; UCF = unilateral club foot; AFDdipj/ UCF = UCF foals earlier seen with AFDdipj; Percentages are of the sample population in each year except AFDdipj/ UCF which is percentage of original AFDdipj.

The AFDdipj cases were first noted as occurring from February 28 to July 4 and peaked in May with 28 cases. The incidence of AFDdipj by days of the year was not significant (Anderson-Darling, s.d. = 29.43, P = 0.207). 72 UCF cases occurred in the population: they were first noted from April 4 to October 19 and peaked in June with 28 recorded. The incidence of UCF by days of the year was very significant (Anderson-Darling, s.d. = 42.33, P < 0.01) *(Figure 5).*

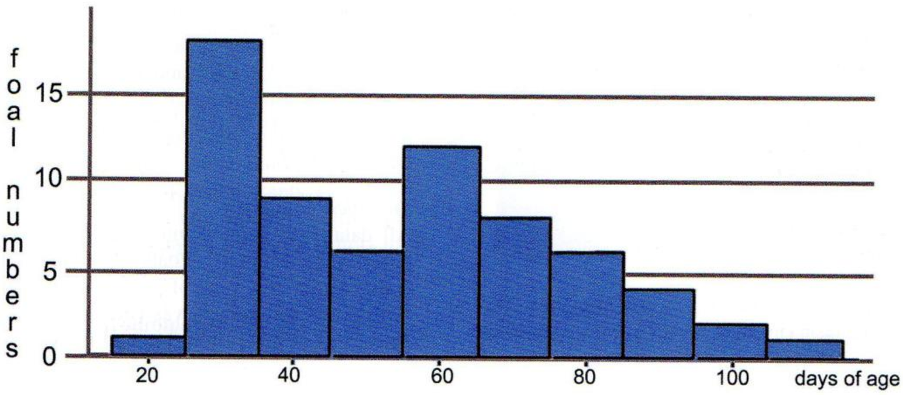

Fig. 3: *The age in days, of the foal when an AFDdipj was first recorded.*

The number of foals which had been noted as AFDdipj (67) which were later noted as UCF was 23. There was a highly significant association of AFDdipj to UCF (χ^2_1 = 11.8, P < 0.001). The average time from an AFDdipj to UCF had a mean of 48.86 days ± s.d = 35.83. Of the 318 healthy foals, 49 were recorded as UCF (16%) (Table 1).

The number of foals with UCF was; UCF LF = 18, UCF RF = 54. As a percentage of the sample population (n=373); UCF LF = 4.82%, UCF RF = 14.47% of the population and as a percentage of UCF 75%:25%. This is a highly significant association (χ^2_1 16.06, P < 0.001) of UCF with leg.

The number of foals that develop an AFDdipj varied according to the month in which they were born, with the largest number of cases, 21, in March. The percentage increased, from January (12.5%) to peak in April (25.3%), before dropping in May to 10%. There was a trend of foals born earlier in the season being less likely to be recorded as AFDdipj, which was not statistically significant (χ^2_4 = 6.302, P = 0.178) (Figure 6). There was a trend of foals born earlier in the season being more likely to develop UCF, which was not statistically significant (χ^2_4 = 7.671, P = 0.104) *(Figure 7)*.

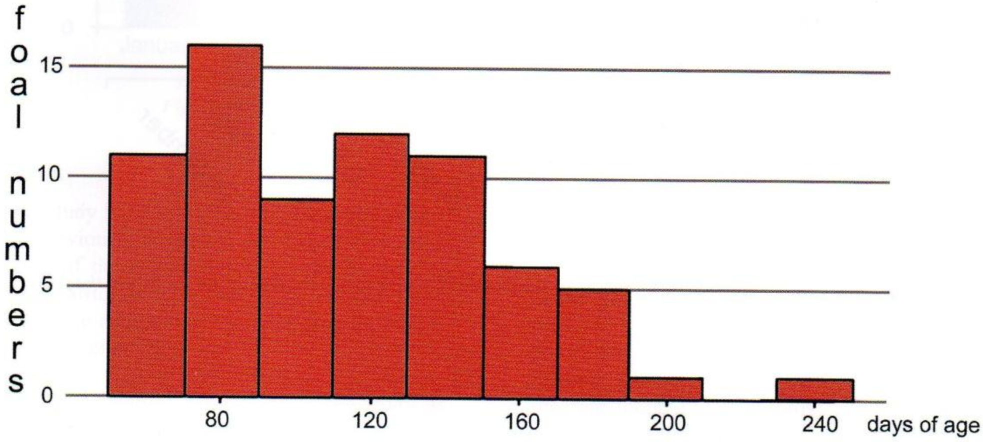

Fig. 4: *The frequency in days of age that foals were first recorded as UCF.*

Discussion:

The figure of 31% of foals having an AFD at some point in their first year of age (Table 1) was on the face of it, a large percentage which would concern most breeders. However, it needs to be understood that the classification of AFDdipj starts with a barely perceptible heel-gap and UCF grading starts at a minimum of only 3° difference in left and right dorsal hoof wall angle. Authors such as McGreevy and Rogers [12] and Heel et al [13] do not use the term "club foot" but only refer to "uneven feet." The term "club foot" is emotive and most authorities not using the Redden grading system would not term a grade 1 CF as such but would refer to "uneven feet".

The percentage of UCFs in the population was 19% which compared to 16% of Tanaka et al [10] and was tested for difference (Fisher's exact test: P = 0.150). This established that there was no difference of prevalence of UCF in the population groups.

Foals affected by AFDdipj were first seen between 20 and 110 days of age. They were first recorded at a median of 54 days and a mean of 53.5 days. Previous authors have given a range of time during which the incidence of AFDdipj occurs, from one week [6] to 12 months [8]. It could be argued that any foal seen with an AFDdipj at one week of age is a congenital flexural deformity (CFD). The usual definition of CFD is that it is seen post partum or immediately after, when the foal is standing and walking.

The age range at which AFDdipj occurs was similar to Trotter [7], who suggested that they occur between one month and four months of age. This author, together with Adams and Santschi, [4]; Fackelman, [6]; Bramlage, [8]; Kidd and Barr, [5] gave no suggestion that they based their opinion of AFDdipj incidence on data. For this study the data was collected on a first-noted basis and therefore the quoted range of 20 - 110 days does not mean that AFDdipj was not seen beyond 110 days.

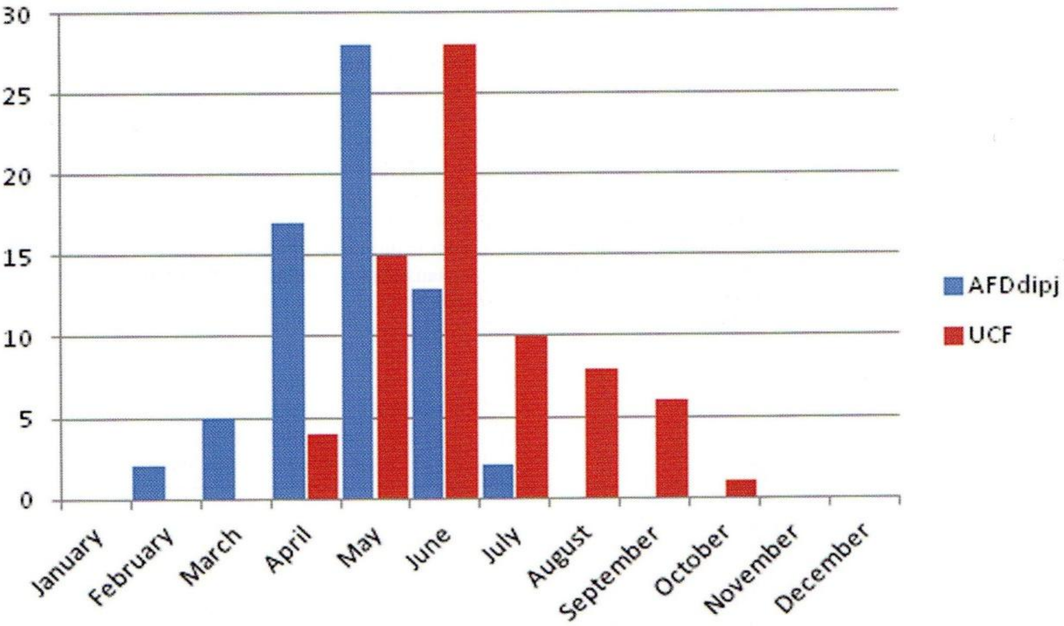

Fig. 5: The incidence of AFDdipj and UCF by the months in which they were first recorded during the year

The incidence by age of the AFDdipj cases may give a clue as to the cause or causes. The two main theories are said by Kidd and Barr [5] to be; a) a mismatch in bone and tendon/ligament growth, and b) contraction of the muscotendinous unit in response to pain. These authors dismiss a) by suggesting that the timing of an AFDdipj is six weeks to six months and therefore well beyond the rapid growth phase of the lower growth plates which, they say, peaks at about two months. This study has shown that the time range of the condition was narrower and ends earlier than that suggested by Kidd and Barr [5] and has been found to be between 20 - 110 days (Figure 3). The result of this survey for age of AFDdipj incidence was very significant, with a mean of 53.5 days of age, ± 22.7 (s.d.) and therefore very close to the peak of the rapid growth phase, which Barr and Kidd give as two months of age [5]. The second suggestion (b) that AFDdipj occurs as a response to pain was explained by describing a horses' reaction to many painful stimuli such as hard ground, causing a lack of weight-bearing and contraction of the muscular portion of the musculotendinous unit.
However, this study showed that the incidence of heel-up stance of AFDdipj occured before hard ground is usually experienced in the UK. In addition, during the months that usually have hard ground (July, August) only 3% of cases occurred. This could be the subject of further study.

In addition, when a horse of any age is chronically lame in one limb and therefore either non-weight-bearing or bearing less than normal weight, the hoof contracts and becomes more tubular in shape. The heels are narrower than normal for the size of foot and the frog is atrophied. This is not the same shape as is seen with a CF, which often has wide heels and a solar outline more akin to a hind foot. It may be that "*rapid bone growth may contribute to orthopaedic pain,*" Kidd and Barr [5] and that the muscle contraction is a response to it.

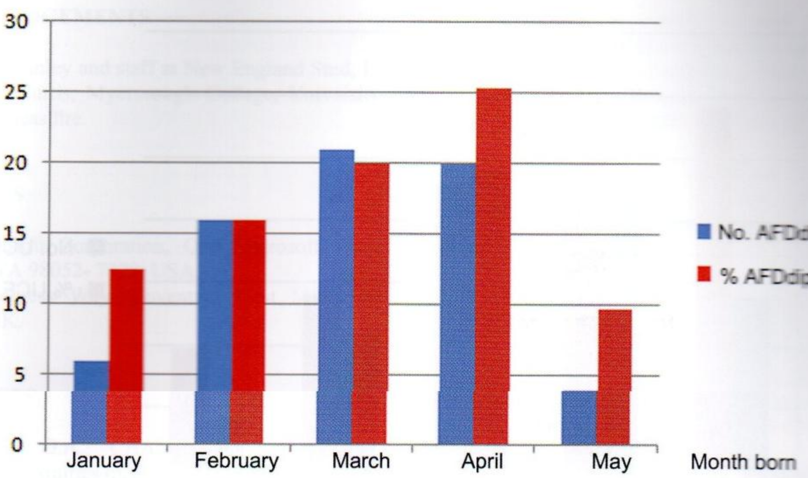

Fig. 6: *The incidence of AFDdipj by the months in which the foals were born.*

Because this study has shown that this condition occurs earlier than previously suggested and close to the rapid growth phase of the distal physes, it may be time to reassess the earlier theory that it is caused by a mismatch in bone and tendon/ligament growth. This is a subject for further study.

72 foals (20% of sample) were noted in the study as UCF and none were seen with this condition before 53 days in age. The oldest was 242 days but had not been seen before, having arrived at the stud for sales preparation. This explainable anomaly apart, the oldest that a foal was first noted was 197 days. There were seven foals first noted as UCF between 175 and 197 days which, on re-checking the data, each had been seen regularly a number of times before without being assessed as UCF. The authors are therefore confident that the condition did arise around this time. No foals in this study were seen with either an AFDdipj or UCF in the hind legs. This confirmed the findings of Tanaka *et al* [11].

AFD had a seasonal incidence with AFDdipj recorded from February to July and UCF from April to October *(Figure 5)*. It is possible that seasonal factors such as sunlight, weather, ground condition, exercise, or nutrition are not contributing to AFD, but that these two patterns are merely reflecting the foaling pattern. All foals were born between January and May and with age being a very significant factor in AFDdipj one would expect to see the foaling pattern mimicked later in the year *(Figure 5)*. It may be possible to separate the effects of age and season by studying AFDdipj and UCF incidence in an appropriately sized population of foals for which the seasonal breeding pattern is not artificially constricted.

In the past, an association linking AFDdipj and UCF was so commonly accepted that they have been grouped together as one condition known as acquired flexural deformity (AFD) without an attempt to separate them. For this study, AFD was divided into the two categories of AFDdipj and UCF, as they were easily identified and differentiated. The results showed that there was a highly significant association between AFDdipj and UCF ($\chi^2_1 = 11.8$, $P < 0.001$) confirming the widely held but untested belief that they are stages of AFD syndrome affecting the DIPJ.

The implications of the time of conversion from AFDdipj to UCF (mean of 48.86 days ± s.d = 35.83) reinforces the need for rapid clinical action. Treatments, such as exercise restriction by box-rest [14] and/or intravenous oxytetracycline [5], need to begin immediately that AFDdipj is recognised.

The ratio of right RF UCF to LF UCF was 5%:14% of the population and as a percentage of UCF 75%:25%. This was a highly significant result ($^2_1$ 16.0556, $P < 0.001$). The authors do not conclude that lateralised behaviour causes UCF. It could be possible that an AFDdipj may cause flexor tendon tension in both front legs but then lateralised behaviour affects the dominant (greater weight-bearing) leg while protecting the protracted (lesser-weight-bearing) foot and the foal then becomes UCF. This is an area for further study.

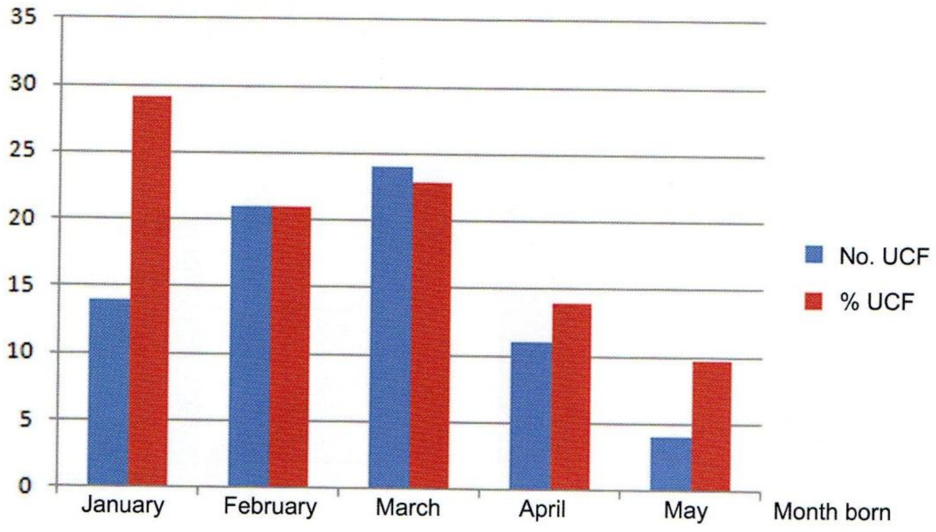

Fig. 7: The incidence of UCF by the months in which the foals were born

The percentage of AFDdipj cases varied according to the month in which the foal was born *(Figure 6)*. There was a rising trend which, because the number and percentage drops in May, was not statistically significant (χ^2_4 = 6.302, P = 0.178). This pattern was unexpected. The authors would suggest that it may be linked to exercise and quality of nutrition but this does not explain the sudden reduction in numbers and ratio in May, when one might expect turn-out time and size of paddock to be similar to April.

A typical treatment for AFDdipj is to restrict exercise and often in unresponsive cases, the foal and mare are given total box-rest. It is often over-looked that box-rest restricts grass intake as well exercise. It may be that early foals are protected from excess nutritional intake because the grass is a lower quality and that by May the spring grass has diminished in quality and quantity due to the weather and the larger number of horses per paddock. Further study is needed to understand this trend.

Although the largest absolute numbers of UCFs were foaled in March; when seen as a percentage of the foals born in each month, January had the highest percentage of UCF per month at 29% *(Figure 7)*. The smallest number (4) and percentage (10%) occurred in May. It appears that early born foals were more likely to have UCF. This trend was almost the converse of the AFDdipj cases by their month foaled. It is difficult to explain these two contrasting findings. A foal born in January had only a 12.5% chance of being AFDdipj later in the year but a 29% chance of later having a UCF. Further studies are required to answer the question of why a foal born in a month that produces almost the lowest chance of an AFDdipj also has the highest chance of UCF. It is possible that the January foals with AFDdipj were less obvious and therefore developed a UCF unseen. The opposite may be true of April foals where 25% were likely to develop an AFDdipj but only 14% become UCF. This could be because they were more readily identified and the necessary treatments and management changes were undertaken. However, treatment and management of AFDdipj and UCF were not the subject of this paper.

CONCLUSIONS

The following conclusions are drawn from this work by the authors :

1. The incidence of AFDdipj in the sample population was 18%.
2. The incidence of UCF in the sample population was 19%.
3. In cases of AFDdipj, incidence by age was very significant, occuring between 20 and 110 days of age.
4. AFDdipj had a trend towards seasonal incidence (February to July) which was not significant.
5. There was a highly significant association between AFDdipj and UCF and a foal with AFDdipj had a 33% probability of developing UCF.
6. The time of conversion of AFDdipj to UCF had a mean of 48.86 ± s.d. 35.83.
7. UCF was a predominantly right sided condition which was highly significant.
8. There was a trend for foals born earlier in the season being more likely to develop a UCF, which was not statistically significant.

OWNER CONSENT

The stud farm owner gave explicit consent for the study and publication.

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MATERIALS

Excel, Microsoft Corporation, One Microsoft Way, Redmond, WA 98052- 7329, USA.
Minitab, Progress Way, Coventry, West Midlands, CV3 2TE, UK.

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