

Regional variation in digital cushion pressure in the forefeet of horses and elephants.

Horses and elephants have extreme foot designs; horses have an unguligrade foot posture with small, single-toed, rigid hooves housing a small fibrous digital cushion, whereas elephants have large, multi-toed, functionally plantigrade, compliant feet with large adipose filled digital cushions. The morphology of the digital cushion is divergent in these species, in terms of its size, shape, volume, composition and organisation. In the context of foot-ground contact this is interesting, because feet nonetheless have to perform similar mechanical functions in all terrestrial species. How well the digital cushion functions under load may contribute to the aetiopathogenesis of foot disease; a sub-optimal digital cushion is less likely to distribute (and thus reduce) high pressures, moderate impact shock and vibration, or prevent unwarranted bone displacement.

In this study, we seek to understand how the digital cushion morphologies evident in horse and elephant feet influence internal and external foot pressures. Our novel use of invasive blood pressure monitoring equipment, combined with a pressure pad and force plate, enabled measurements of (*ex vivo*) digital cushion pressure under increasing axial loads in seven horse and six elephant forefeet. Linear mixed effects models (LMER) revealed that internal digital cushion pressures increase under load and differ depending on region; elephant feet experienced higher magnitudes of medial digital cushion pressure, whereas horse feet experienced higher magnitudes of centralised digital cushion pressure. Direct comparison of digital cushion pressure magnitudes in both species, at equivalent loads relative to body weight, revealed that medial and lateral pressures increased more rapidly with load in elephant limbs. Within the same approximate region, internal pressures exceeded external, palmar pressures (on the sole of the foot), supporting previous Finite Element (FE) predictions. High pressures and large variations in pressure may relate to the development of foot pathology, which is a major concern in horses and elephants in a

captive/domestic environment.

We suggest that heterogeneity within the digital cushion allows it to perform conflicting mechanical functions during locomotion; different digital cushion regions may be responsible for specific functions and therefore feature appropriate properties to do so. Determining how internal structures such as the digital cushion respond to locomotor loading is essential to understanding foot health and pathology, as well as the functional consequences of evolutionary changes in foot morphology.

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15 Introduction

16 Digital cushions are specialised fatty/fibrous pads (Wearing and Smeathers 2011) that protect
17 bony prominences within the fore (manus) and hind feet (pes). As well as absorbing shock at foot
18 impact, it has been proposed that these structures may distribute and thus reduce high, localised
19 pressures (and loads) which can cause damage if their magnitudes exceed tissue safety
20 thresholds. Mammalian digital cushion morphology shows a remarkable amount of variation
21 (Egerbacher *et al.*, 2005; Weissengruber *et al.*, 2006; Raber *et al.*, 2004; Chi 2005; Ker 1999; Hsu
22 *et al.*, 2007). Within the context of foot-ground contact, this is interesting because feet have to
23 perform similar mechanical functions such as generating pressures against the substrate to
24 support and propel the body (Alexander *et al.*, 1986; Chi and Roth 2010). Pressure is equal to
25 force divided by area; therefore pressure can be moderated by reducing the force applied, or by
26 increasing the surface area over which the force is applied. Although foot surface area increases
27 with body mass, it may do so at a disproportionate rate, meaning that foot pressures are higher in
28 larger mammals (Michilsens *et al.*, 2009). This study seeks to understand how extreme foot
29 design influences internal and external foot pressures.

30 Horses and elephants are very similar in some respects; both are large, parasagittally striding,
31 fairly straight-legged terrestrial quadrupeds, with long daily travel distances and grossly similar
32 habitats (Carrano 1999; Hutchinson *et al.*, 2009). And yet, these two mammals have extremely
33 different foot and digital cushion morphologies. Horses, as ultimate examples of unguligrades,
34 have a fully erect foot posture whereby the most distal phalangeal bone supports body weight via
35 the hoof (Douglas *et al.*, 1998; Bowker 2003); the carpal (or tarsal) bones, metapodials and
36 phalanges are permanently raised off the ground (Fig. 1A). Their relatively small digital cushions
37 (~13 % of total forefoot volume, Warner, 2014), have no discernible compartmentalisation and
38 are contained within a rigid hoof capsule. Horse digital cushions are almost devoid of adipose
39 tissue, instead containing dense connective, myxoid tissue and fibrous cartilage (Egerbacher *et*
40 *al.*, 2005; Wilhelm *et al.*, 2005).

41 The opposing postural extreme is termed plantigrady, whereby the carpal (or tarsal) bones,
42 metapodials and phalanges lie along the ground and with a system of digital and metacarpal/tarsal
43 cushions, support body weight (Fig. 1B). Elephants are unusual in that they have a sub-
44 unguligrade skeletal foot posture, but their digital cushion renders them functionally plantigrade,
45 because the digital cushion (containing an enlarged strut-like “predigit”) directly supports body
46 weight (Miller *et al.*, 2008; Hutchinson *et al.*, 2011). Their substantial digital cushions extend
47 proximally from the sole to the metacarpals, providing almost 40 % of the foot’s total volume
48 (Warner, 2014). Elephant digital cushions are formed of chambers of differing geometry filled
49 with adipose tissue, with collagen, reticulin and elastic fibers also present (Weissengruber *et al.*,
50 2006).

51 How do these divergent digital cushion (and foot) morphologies mitigate high local pressures
52 such as those experienced during foot impact or full weight support? Does the material
53 composition of elephant digital cushions lead to greater compliance and viscosity compared to

54 horse digital cushions, meaning that they are better adapted to distributing and thus minimising
55 high pressures (Panagiotopoulou *et al.*, 2012) and dampening impact forces (Aerts *et al.*, 1996;
56 Warner *et al.*, 2013)? And do supposedly disproportionate external foot pressures present a
57 challenge to large animals with regards to foot health? As a first step towards these broader goals,
58 here we focus on these two mammals in order to investigate how internal pressures change in
59 horse and elephant digital cushions under load. Our study asks three principal questions, as
60 follows.

61 First, does axial limb loading cause internal digital cushion pressures to change? Importantly, it is
62 still unclear whether digital cushion pressures do change under load, due to the experimental
63 difficulties associated with quantifying internal pressures. Invasive measurements of force are
64 limited for ethical and practical reasons (e.g. animals might be least likely to move normally if
65 their feet are subjected to surgical procedures), and despite finite element models indicating
66 regional distributions of stress (Spears *et al.*, 2005; Luo *et al.*, 2011), these models are limited
67 due the oversimplifications necessary to conduct the analysis. Considering that material testing of
68 isolated (human heel pad) tissue samples have revealed variable results with regards to
69 mechanical properties (DeClerq *et al.*, 1994; Aerts *et al.*, 1995), this study quantifies internal
70 pressure (*in situ*) using *ex vivo* loading experiments of cadaveric forefoot material.

71 Previous work on horses *in vivo* (Dyhre-Poulsen *et al.*, 1994) suggested that limb loading induces
72 negative digital cushion pressure (i.e., suction), however this study experienced substantial
73 technical difficulties with transducer function. An increase of pressure induced by compression
74 may be countered to some degree by the effects of medio-lateral and dorso-palmar foot expansion
75 (Taylor *et al.*, 2005; Thomason *et al.*, 1992); however, the foot seems unlikely to expand to the
76 extent needed to create negative pressure. For example, we calculated that if a load of 2500 N
77 was applied (~50 % body weight), the digital cushion's cross sectional area would have to
78 increase by > 5000 % to drop below zero pressure, relative to the unloaded limb. This suspicion
79 provided additional motivation to re-examine horse digital cushion pressures in this study.

80 In both horses and elephants, the dorsal portion of the forefoot is contained-- either by the hoof
81 capsule in horses or by the digits in elephants. Curiously, human heel pad (finite element) studies
82 found that confining the heel pad reduced the predicted internal stress (Spears *et al.*, 2007). The
83 palmar portion is, by comparison, more free to deform; hence digital cushion pressures in this
84 region are more likely to be influenced by the extent of deformation. The primary hypothesis for
85 this study is that internal digital cushion pressure will increase under load; further, because
86 elephant digital cushions are likely to have greater compliance compared to horse digital
87 cushions, equivalent loads (in terms of % body weight) will induce lower magnitudes of pressure
88 change in elephant feet.

89 Second, do regional pressure differences exist within the digital cushion of horses or elephants?
90 Previous studies on human heel pads indicate that the chambers present behave like fluid-filled
91 sacs (Ker 1999); compressive loads cause pad deformation leading to displacement of adipose
92 tissue, and tension in the fibrous septa between chambers and surrounding skin (Spears *et al.*,
93 2007). If the tissue within the chambers acts as an incompressible fluid, assuming constant
94 volume, the pressure within each chamber should be uniform (Pascal's theory), assuming there is
95 no fluid flow between the compartments (Jahss *et al.*, 1992).

96 Considering that elephants have differing chamber geometry (Weissengruber *et al.*, 2006), it is
97 hypothesised that loading will cause differential pressure increases throughout the digital cushion

98 (specifically, macro-chamber pressures will increase with load at a lower rate than micro-
99 chamber pressures). This is because the high ratio of fibrous septa: adipose tissue evident in
100 micro-chambers (in contrast to macro-chambers) is likely to provide greater resistance to
101 deformation (Hsu *et al.*, 2007). It is expected that pressures in these micro-chamber regions
102 (which appear to be located more superficially from visually assessing high resolution MRIs,
103 Warner, 2014, Fig, 2B) increase when small loads are applied, whereas pressures in macro-
104 chamber regions will remain more stable until larger loads are applied.

105 Although horses lack discrete digital cushion chambers (visual assessment of high resolution
106 MRIs, Warner, 2014, Fig. 2A), loading is expected to cause differential pressure increases
107 throughout the cushion. Specifically, we hypothesise that pressure will increase to a greater
108 degree in areas closest to the cushion's boundaries and in areas closer to points of compression.

109 Finally, how do external palmar and internal digital cushion pressures (in the same relative
110 region) compare? Data from finite element models have predicted that internal pressures exceed
111 external pressures (Spears *et al.*, 2007; Luo *et al.*, 2011), but this has not been determined
112 experimentally. Here we compare synchronous pressure pad measurements of palmar pressure
113 and internal digital cushion pressure measurements in cadaveric forefeet material for both study
114 taxa.

115 **Materials and Methods**

116 Cadavers

117 A detailed list of subject information is in Table 1. Forelimbs, as opposed to hind limbs, were
118 selected because forelimbs are under greater load in both species (Manter 1938; Jayes and
119 Alexander 1978; Ren *et al.*, 2010; Warner *et al.*, 2013). Three horse (*Equus caballus* Linnaeus
120 1758) forelimbs were obtained from a local abattoir (Holts Purveyors of Fallen Stock, Stanstead
121 Abbots, UK) and four more were obtained from the Royal Veterinary College's (RVC) Equine
122 Hospital (post-mortem specimens). All had been euthanised for reasons other than
123 musculoskeletal disease and unrelated to this study. All horses were adult, of mixed breeding,
124 body masses 552 ± 95 kg (mean \pm S.D.), 15 ± 10 years old. Limbs were frozen immediately after
125 disarticulation and stored at -20°C until they were thawed ready for use. Limbs were prepared as
126 described by McGuigan and Wilson (2003). Shoes were removed if present and hooves were
127 minimally trimmed (excess hoof wall was rasped level with the sole). Thawing took between 24-
128 48 hours and all limbs had been at room temperature for at least six hours prior to data collection.

129 Elephant forelimbs were obtained from various zoo/safari park sources within the European
130 Union (specimens from Hutchinson *et al.*, 2011). Four adult Asian elephant (*Elephas maximus*
131 Linnaeus 1758) and two adult African elephant (*Loxodonta africana* Blumenbach 1797) limbs
132 were obtained; estimated body masses 3532 ± 1052 kg (mean \pm S.D.); 36 ± 16 years old.
133 Elephants had died naturally or been euthanised for various health conditions; three limbs had
134 obvious gross pathologies in the manual bones, but these had no apparent qualitative influence on
135 the response of the digital cushion (see Data S1 for details of gross pathologies, Fig. S2, S3 for
136 details of individual variation in elephants; Fig. S4, S5 for details of individual variation in
137 horses). There is no evidence in the current literature of differences in digital cushion
138 morphology or properties in Asian or African elephant forefeet; we assume that both species are
139 far more similar to each other than to horses in these parameters. All limbs had been
140 disarticulated at the level of the carpus and had been stored at -20°C until they were thawed
141 ready for use. Laboratory temperature was approximately 16°C throughout the experiments;
142 humidity was not controlled.

143 Equipment

144 Internal pressure was quantified using invasive blood pressure monitoring apparatus equipped
145 with four transducers (0.2 Hz; Logical®, Smiths Medical, Kent, UK). Data were displayed on a
146 monitor (Datex Ohmeda, Southwest Medical Ltd, Bedfordshire, UK) and logged using custom-
147 written Datex Logger software (v.1, Kyle Roskilly). External (palmar) pressure was measured
148 using a pressure pad (25 Hz; 0.5m RS Footscan plate containing 4096 sensors, plus RS Footscan
149 Gait Software; Olen, Belgium), while applied force was measured using a Kistler force platform
150 (25 Hz; type 9287B, Kistler Instruments AG; Winterthur, Switzerland), plus custom written
151 Labview software (v2009 SP1, National Instruments; Austin, TX). Limbs were loaded using a
152 manually operated hydraulic loading ram (10 tonne capacity, Clarke Strong-Arm, Dunstable,
153 UK), mounted to a custom built loading frame. The frame was securely bolted to the laboratory
154 floor, which housed the force platform and pressure pad.

155 Needle placement

156 The (internal) pressure system was validated using the SURE-CAL® integrity facility on each
157 transducer. Individual lines were flushed with water to ensure they were free from air bubbles,

158 prior to needle insertion. Figs. 3, 4 show the intended pressure sampling (needle) locations in
159 horse and elephant limbs respectively. Four BD spinal needles (quince type point, 18 GA 3.50 IN
160 1.2 x 90 mm) were used, and additionally a BD Angiocath™ needle (14 GA 5.25 IN 2.1 x 133
161 mm) was used for the deepest location in the elephant. Needle placement was verified using
162 either radiographs (dorso-palmar 65 kV, 24 mA; lateral 70 kV, 24 mA, using a PX-15H from
163 SMR Medical Imaging, UK) of horse feet, or CT scans (5 mm axial slice thickness, 140 kV, 200
164 mA, using a Lightspeed QX/i from GE Medical, UK) of elephant feet.

165 Limb loading

166 In horse limbs, a 12 mm diameter hole was drilled through the elbow joint; in elephant limbs, the
167 hole was drilled through the distal carpal bone row, into the third/fourth metacarpal. The loading
168 ram's pin was inserted into the pre-drilled hole, and then foot placement was adjusted until
169 loading generated a centre of pressure (CoP) that was central to the foot (assessed using the
170 pressure pad, see Fig. S6 showing the experimental set up and Fig. S7, S8 for the location of the
171 CoPs in each individual across all trials). Additionally, in the horse, the foot was positioned so
172 that the metacarpal became vertical under load, following McGuigan and Wilson (2003). Once
173 the pin was inserted, the ram's head rested on the surrounding bone, preventing the pin from
174 driving deeper into the bone.

175 Pressure measurement adjustments

176 Drift in the pressure channels was monitored for at least 10 minutes once needles were *in situ* and
177 the foot was positioned centrally beneath the loading ram. Trials began once drift did not exceed
178 ± 0.05 mmHg s^{-1} . To overcome differences in static pressure, all channels were zeroed; the
179 measured change in internal pressure as a result of loading was therefore measured relative to
180 zero pressure (enforced by zeroing, in the unloaded state via the Datex Ohmeda hardware).

181 Trials

182 Each 12 minute trial consisted of three phases; baseline data were recorded during the initial five
183 minutes, after which the limb was loaded (which took up to ~ 30 seconds, depending on the
184 magnitude of the load). The limb remained loaded for two minutes, after which it was unloaded;
185 data were then recorded for a further five minutes. Applied loads spanned 0-150% body weight
186 (BW) in horse limbs and 0-60% BW in elephant limbs; total number of loads per limb did not
187 exceed 20. Trial timings ensured the limb had at least 10 minutes rest in between applied loads.
188 The lengthy nature of applying the load (via the manually operated ram), meant that the capture
189 frequency of the pressure pad had to be set to 25 Hz (trial lengths were restricted to 1000 data
190 points), and thus the force platform's recording frequency was set to match the pressure pad's.
191 Since the ram could not replicate high physiological loading rates, the analysis focussed on
192 internal and external pressure changes as a result of static loads only (i.e., data collected during
193 loading/unloading did not form part of the analysis).

194 Data Processing

195 All data were exported to Matlab (v.R2001b, Mathworks Inc, Natick, MA, USA) for custom
196 analysis; raw (Kistler) force data were low-pass filtered (100 Hz single pole RC filter), zeroed
197 and summed to get F_x , F_y and F_z , although only vertical force (F_z) was used here. The pressure
198 pad was calibrated with a person of known mass (as per the manufacturer's instructions);
199 however, our preparatory tests showed that the pad did not consistently quantify the applied

200 force. A force correction value was therefore calculated using the force platform data to correct
201 the force (and pressure) data from the pressure pad (see Data S9 for further details). Spatial
202 registration of external pressure data was not necessary because the foot's position on the pad did
203 not change in between trials.

204 Figure 5 shows the internal pressure data (recorded in digital cushion four locations) and the
205 vertical force data for a typical trial: a horse limb loaded to 112 % BW (~6000 N). Both mean
206 and maximum instantaneous pressures were recorded because previous studies have shown that
207 both are important in pathological foot conditions (Jahss *et al.*, 1992). Mean internal digital
208 cushion pressure change was calculated by subtracting the average pressure data (calculated over
209 a 30 second period, grey shaded area in Fig. 5) before loading, from the average pressure data at
210 full load. The maximum instantaneous internal pressure was identified as the highest peak in
211 pressure following load application. Peak and average vertical forces as well as the rate of force
212 application were also calculated and recorded.

213 In order to compare internal and external pressures in the same region, the coordinates of the
214 needle tips within the digital cushion were obtained via radiographs or CT scans; the z
215 coordinates (relating to proximal height) were recorded as a percentage of the height of the distal
216 phalangeal bone in the horse, and as a percentage of the height of the proximal phalangeal bone
217 in the elephant (digit III). For horse limbs, radiographs and external (palmar) pressure images
218 were overlaid to identify x,y coordinates (relating to medio-lateral width and dorso-palmar length
219 respectively (Fig. 6). Image J (1.45s/Java 1.6.0_20, National Institute of Health, USA) software
220 was used to obtain the z coordinate. For elephant limbs, CT data were imported into Mimics
221 software (v16.0, Materialise Ltd, Leuven, Belgium), where the needles and sole were
222 reconstructed in 3D, before the model and the corresponding external (palmar) pressure image
223 were overlaid to obtain x,y,z coordinates (Fig. 7). Mean external (palmar) pressures at the same
224 x,y location as the needle tips were calculated from averaging the external pressures from the
225 nine closest sensors over a period of 10 seconds (50 frames) at full load.

226 Statistical Analysis

227 Linear mixed effect models (LMER, in R Programming Language (v.2.14.0); [http://www.r-](http://www.r-project.org/)
228 [project.org/](http://www.r-project.org/)) were used to analyse the data. Although plotting load against pressure may have
229 shown a quadratic relationship (due to digital cushion viscoelasticity), the addition of a quadratic
230 term to the linear model did not make a significant contribution to model fit in either species. The
231 quasi-static nature of load application was likely to negate any rate-dependant behaviour;
232 therefore quadratic terms were omitted from the model:

233 Model = LMER(pressure (Nm⁻²) ~ load (N) + (1 + load (N) | individual))

234 The fitted model included load (in Newtons, or as a % BW) as the fixed effect, and terms that
235 allowed the intercept and linear term (slope) to vary by individual (Figs. S2-S5 show individual
236 variation). To determine the significance of the fixed effect estimates, *P* values were generated,
237 while comparisons between regions and comparisons between species were achieved using
238 ANOVA and post hoc (Bonferroni) tests. Significance was set at $P < 0.05$.

239 Results

240 Individual forefeet were loaded a maximum of 20 times during the period of data collection; this
241 provided four measures of internal pressure per load, per limb, totalling 339 observations in horse
242 limbs ($n = 7$; see Fig. S2, S3 for individual variation) and 252 observations in elephant limbs ($n =$
243 6 ; see Fig. S4, S5 for individual variation). Replicating precise needle placement was difficult to
244 achieve across individuals due to differences in foot size and conformation (Fig. 8 shows a
245 relatively upright (A) versus flat-footed (B) conformation in two horses). Internal pressure data
246 were therefore grouped by region; 1) deep central (d.c.), 2) superficial central (s.c.), 3) superficial
247 medial (s.m.) and 4) superficial lateral (s.l.). Figure 9 shows normalised needle locations; i.e., as a
248 percentage of foot width, length and distal phalangeal bone height in the horse or proximal
249 phalangeal bone height in the elephant (digit III).

250 Internal Pressure

251 The positive linear terms generated by the LMER analysis show that in both study taxa, and in all
252 regions analysed, internal digital cushion pressure increased with load (Table 2). Excluding the
253 deep central region in horse limbs, intercepts were not significantly different from zero. Mean
254 and maximum instantaneous pressures in each region tended to follow a similar pattern. In horse
255 limbs, increasing load had the greatest effect on pressures in the superficial central region (Fig.
256 10A). Conversely, in elephant limbs, increasing load had the least effect in the superficial central
257 region (Fig. 10B). Our results revealed that horse limbs experienced higher starting magnitudes
258 of pressure in the deep central region (i.e., both intercepts, for mean and maximum instantaneous
259 pressure, were significantly different from zero, $P < 0.05$).

260 Of the four digital cushion locations analysed, some pressures differed depending on region
261 (LMER, ANOVA, $P < 0.05$, Tables 3 and 4). Post hoc analysis (Bonferroni) showed that in both
262 species, central pressures were similar regardless of needle depth ($P > 0.05$); furthermore,
263 superficial central pressures differed from superficial medial pressures ($P < 0.05$) in both species.
264 Elephant digital cushion pressures were slightly more uniform (i.e., less variable) than horse
265 digital cushion pressures.

266 Under equivalent loads (in terms of % BW), the superficial medial and lateral regions in elephant
267 digital cushions experienced higher magnitudes of internal pressure when compared to medial
268 and lateral regions in horse limbs (Fig. 11, ANOVA, $P < 0.05$). Despite horse limbs experiencing
269 higher starting magnitudes of pressure in the deep central regions of the digital cushion,
270 increasing load had a similar effect on central pressures in both species (ANOVA, $P > 0.05$).

271 External pressure (at the palmar surface)

272 Substantial technical issues with the pressure pad arose during data collection (see Data S9 for
273 details), and the plate was sent back to the manufacturer for repair. The data reported here are for
274 5 horse and 5 elephant forelimbs. Figs. S7 and S8 show that foot placement generated CoPs that
275 were central to the feet, as per the observations during data collection. The pressure pad is
276 optimised for (clinical) human use, i.e., a 70 kg person loading the pad to ~ 1 -2 times BW during
277 activity. As the maximum capacity of the pressure pad was 200 Ncm^{-2} , pressure values above this
278 threshold were disregarded as unreliable. The remaining external pressures that we measured
279 have the same order of magnitude as previously reported values (Table 5); at standing forelimb
280 loads (~ 30 % BW), mean external palmar foot pressure was higher in horse feet compared to
281 elephant feet (although there were insufficient data to test this statistically).

282 The palmar surface area (SA) in elephant forefeet was around seven times larger than the palmar
283 SA of horse forefeet (Table 6), whereas elephant body masses were around six times larger (Table
284 1). At the level of the needle tips (i.e., where internal pressure was sampled), the digital cushion's
285 cross-sectional area (CSA) constituted one-fifth and one-third of the total palmar foot SA in
286 horses and elephants respectively.

287 There was only a weak correlation between applied load and external palmar pressure ($r=0.36$
288 and $r=0.31$ in horses and elephants respectively); the distribution of pressures over the palmar
289 surface was more variable in elephant forefeet (Table 6). Internal digital cushion pressures were
290 consistently higher than external palmar pressures in the same (x,y) location (Mann Whitney U
291 Test, $P<0.05$), and there was minimal correlation between internal and external pressure ($r<0.25$).

292 **Discussion**

293 We aimed to characterise loads inside and outside the forefeet of horses and elephants in order to
294 compare the influence of extreme digital cushion (and foot) morphology on pressure magnitudes
295 and distributions. Our results reveal that (*ex vivo*) axial limb loading causes internal digital
296 cushion pressure to increase, and regional pressure differences exist within the digital cushion.
297 Further, under equivalent loads (in terms of % BW), elephant feet experience higher medial and
298 lateral pressures than horse feet do.

299 Previous studies have inferred that the lateral portion of the elephant manus experiences greater
300 palmar pressures (Panagiotopoulou *et al.*, 2012) and perhaps bone or tendon stresses (Miller *et*
301 *al.*, 2008). Yet we find that the highest internal pressures are located in the medial (and then
302 lateral) regions of the digital cushion. It is conceivable that the *ex vivo* loading set-up or limb
303 conformation abnormalities induced asymmetrical loading at the level of the foot (Luikart and
304 Stover 2005). However, this would have been reflected in the location of the CoPs (Figs. S7 and
305 S8 show CoPs were consistently central). As a potential explanation for these results, we
306 speculate that high medial pressures are caused by the prepollex (see Hutchinson *et al.*, 2011)
307 directly transferring load to the digital cushion.

308 Internal chamber geometry and distribution remains undetermined in elephant digital cushions. In
309 human heel pads, the presence of chambers is associated with preventing bulk flow and gross
310 distortion (Ker 1999); this additional information could help to explain digital cushion behaviour
311 in elephant limbs under load. On the basis of the variation in digital cushion pressure shown here,
312 we propose that material properties differ throughout the digital cushion due to differences in
313 structural organisation. The medial (and perhaps lateral-) portions of the digital cushion in
314 elephants experience higher pressures; this may be due to greater fibrous tissue content and/or
315 smaller chambers (Hsu *et al.*, 2007). This would also fit with the observation that their feet land
316 with a slightly lateral CoP trajectory (Panagiotopoulou *et al.*, 2012). The expectation that pressure
317 in the deep central digital cushion region remains more stable until large loads are applied was
318 not fulfilled; although the assumption that this expectation was based on is likely to be correct
319 (macro-chambers have greater initial compliance in human heel pads, Hsu *et al.*, 2007). MRI-
320 guided needle insertion would be required to confirm needle placement within specific chambers
321 to investigate this further.

322 In contrast to elephants, in horse forefeet we found that the highest internal pressures are located
323 in the superficial central region. This may be accounted for by the large degree of extension in the
324 DIP joint, causing the middle phalangeal bone to compress this region of the digital cushion
325 (Taylor *et al.*, 2005). Although increasing load has less effect on pressure in the deep central
326 region, we speculate that the higher starting magnitudes of pressure are due to this portion being
327 thinner, denser and more rigidly contained on all aspects. We acknowledge that the locations
328 sampled for pressures in horse and elephant forefeet are not strictly homologous, but both taxa
329 are so radically transformed and insertion of needles into precise locations is so difficult that
330 strict application of topological homology in our experimental setup was not practical.

331 Pressures within the digital cushion were higher in magnitude than the external palmar pressures,
332 consistent with previous finite element models of human heel pads (Spears *et al.*, 2007; Luo *et*
333 *al.*, 2011). The absence of a stronger (linear) correlation between external and internal pressure in
334 the same (x,y) location is not surprising, because previous studies have suggested external forces

335 are poor indicators of internal stresses (Loerakker 2007). In contrast to the negative allometry of
336 plantar foot SA that Michilsens *et al.*, (2009) reported, we found that palmar foot SA in elephant
337 feet increases to a greater extent than body mass. This explains our finding that (mean) palmar
338 pressures were lower in magnitude in elephant feet compared to horse feet. The ratio of palmar
339 foot SA to contact area was 45% greater in horses, supporting the inference that elephants use a
340 greater proportion of the foot's available SA to support load, which could help protect their sole
341 from high pressures. It remains unclear how the concavity of a horse's sole benefits the animal
342 (although this feature appears to be highly variable; Fig. 8); certainly, limiting the contact area is
343 counter-productive to reducing pressure. It is likely that the concavity aids friction and grip
344 (Pardoe *et al.*, 2001), allowing horses to move at faster speeds on loose or soft surfaces, and the
345 reduction of tissue volume it provides surely keeps foot mass low, with consequent energetic
346 benefits for swinging the distal limb (Wilson and Weller 2011).

347 From prior studies (Weissengruber *et al.*, 2006; Egerbacher *et al.*, 2005), it seems likely that the
348 semi-fluid material inside elephant digital cushions would "flow" more easily than the material
349 inside horse digital cushions due to the higher adipose tissue content present in elephant digital
350 cushions. Yet it is unclear how fat content influences digital cushion function; Buschmann *et al.*,
351 (1993) suggested that lower viscosity associated with adipose tissue enhances efficiency of the
352 human heel pad (although they were focused on shock absorption). Bowker (2003), on the other
353 hand, associated adipose tissue with sub-optimal foot function in horses (although again, the
354 focus was on shock absorption). In our study, higher adipose content (in elephant digital
355 cushions) is associated with higher internal pressures (medial and lateral digital cushion regions
356 only).

357 Although quantifying pressure in four regions gives an incomplete description of the internal
358 stress state throughout the cushion, the use of more needles was considered too damaging to the
359 structure and not feasible in the time frame of the study. The capacity of the force platform
360 (~24,000 N) prevented elephant limbs from being loaded to a similar extent as horse limbs;
361 maximal loads in elephant limbs were only 50-60% BW, whereas maximal loads in horse limbs
362 represented >150% BW. The pressure pad was also operating at near-maximum capacity (200
363 Ncm⁻²), and despite correcting the pad's force measurements (using the force platform), much of
364 the external pressure data had to be excluded from the analysis (as reported in the Results section
365 and detailed in Data S9). The *ex vivo* magnitudes of palmar foot pressure are comparable to *in*
366 *vivo* values previously reported (Panagiotopoulou *et al.*, 2012); however, it is surprising that
367 increasing load did not have a stronger linear correlation with external pressure magnitudes. This
368 anomaly can best be ascribed to the pad being optimised for human use (i.e., at much lower
369 loads).

370 Rate of loading is an important consideration in this study, because viscoelastic tissues have non-
371 linear and rate-dependant responses to loading (Ker 1999; Hsu *et al.*, 2007). The manually
372 operated hydraulic ram could not replicate high physiological loading rates. Although some
373 analysis of the digital cushion's viscoelastic response could have been included, its biological
374 relevance would have been questionable, because loading rate (via the ram) was so slow.
375 Applying rapid and large loads (~30 kN for 0.30 second stance time in a fast-moving elephant,
376 Hutchinson *et al.*, 2006) was not feasible with the equipment available and was deemed unsafe.
377 The fixed point application of load differs from physiological loading whereby a dynamic pattern
378 exists; during *in vivo* locomotion, the point of application starts at the posterior of the foot and
379 moves cranially throughout the stance phase (Panagiotopoulou *et al.*, 2012; van Heel *et al.*,

380 2005). This study's purpose was largely comparative and hence a static approach (replicating
381 standing and mid-stance) was judged to be sufficient as well as the most practical option.

382 Although elephant limbs were loaded at a slightly faster rate than horse limbs, previous work has
383 shown that *in vivo* loading rates in elephants exceed those in horses ($\sim 17,000 \text{ N s}^{-1}$ and $\sim 7000 \text{ N s}^{-1}$
384 in elephants and horses respectively at relatively comparable walking speeds; Warner *et al.*,
385 2013). Ker (1996) found slight creep in human heel pads under *ex vivo* test conditions (1.4 kN,
386 using continuous sinusoidal applications of force); however, it was concluded that this creep was
387 reversible and not associated with tissue damage. We assume that digital cushion deformation as
388 a result of loading did not become permanent (causing internal pressures to remain high, Aerts *et*
389 *al.*, 1995; Ker 1996), because post-load pressures returned to pre-loading magnitudes.

390 Although freezing is unlikely to alter the mechanical properties of human heel pads (Bennett and
391 Ker 1990a; Ker 1996), the thawing process and data collection period (at room temperature)
392 could have resulted in some tissue dehydration. Despite moisture levels being known to affect
393 material properties (Bertram and Gosline 1987; Douglas *et al.*, 1996), it was not feasible to
394 monitor or account for dehydration. Limb deformation may have been reduced as a result of
395 increased tissue stiffness (via dehydration) but using fresh specimens was unrealistic, especially
396 with regards to elephant limbs. Furthermore, absolute measurements of *in vivo* stiffness were not
397 our target; thus for comparative purposes the data should be reasonable approximations.

398 There have been no prior internal pressure measurements for elephant feet, and only one in
399 horses (*in vivo*) (Dyhre-Poulsen *et al.*, 1994). Although it is reasonable to expect some
400 differences between *ex vivo* and *in vivo* limb loading studies (because active and haemodynamic
401 mechanisms associated with force attenuation are absent and tissue properties may change post-
402 mortem; Riemersma 1996), our study is nevertheless valuable, providing insight into how
403 internal digital cushion stresses change in response to load. It is interesting to note that in human
404 medicine, pressures of $\sim 4000 \text{ Nm}^{-2}$ require surgical decompression (Wall *et al.*, 2010; Rasul
405 2011); although digital cushion tissue differs from muscle tissue, the internal digital cushion
406 pressures reported here are almost two times as high.

407 Elephant pressures are slightly more uniform throughout the digital cushion; however, describing
408 the digital cushion as acting as a fluid-filled sac is inappropriate, because pressure is not equal in
409 all locations (i.e., Pascal's theory does not apply). We speculate that the variation in pressure
410 throughout the digital cushion is likely to be due to heterogeneities in material properties and/or
411 structural organisation causing differential deformation. Further work will focus on quantifying
412 the effect of load on 3D digital cushion deformation to test the latter speculation. Internal
413 pressure magnitudes exceed external palmar foot magnitudes, matching the predictions of
414 previous finite element studies (Spears *et al.*, 2007; Luo *et al.*, 2011).

415 **Conclusion**

416 Insufficient functional capacity in human heel pads can lead to the development of shock-induced
417 discomfort and injury (Kinoshita *et al.*, 1996). If high pressures can induce pathology (e.g.
418 Panagiotopoulou *et al.*, 2012), based on the magnitudes measured in this study, we propose that
419 the medial (and lateral) bony prominences in elephant feet may be vulnerable. Indeed, two recent
420 studies of rhinoceroses indicated that osteopathologies are more prevalent in the medial digit
421 (Galanteanu *et al.*, 2013; Regnault *et al.*, 2013). If large variation in pressures (indicating poor
422 pressure distribution) induces pathology, based on the variation in pressure measured in this

423 study, we propose that the elephant's external palmar tissues and the horse's internal (digital
424 cushion) tissues may be susceptible to pressure-related pathology. Determining how the digital
425 cushion responds to loading *ex vivo* may highlight potential weaknesses within the foot and assist
426 our understanding of why mechanically induced pathologies develop.

427 **List of Abbreviations**

- 428 BW body weight
429 CoP centre of pressure
430 CSA cross-sectional area
431 CT computed tomography
432 d.c. deep central
433 LMER linear mixed effect model (in R software)
434 MRI magnetic resonance imaging
435 SA surface area
436 s.c. superficial central
437 s.l. superficial lateral
438 s.m. superficial medial

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Figure 1

Schematic diagram of foot posture in horses and elephants.

Horses are unguligrades (A); the most distal phalangeal bone supports body weight via the hoof (Douglas *et al.*, 1998; Bowker 2003); the carpal (or tarsal) bones, metapodials and phalanges are permanently raised off the ground. In contrast, elephants have a sub-unguligrade foot posture (B), however their digital cushion renders them functionally plantigrade, because the digital cushion (containing an enlarged strutlike “predigit”) directly supports body weight (Miller *et al.*, 2008; Hutchinson *et al.*, 2011).

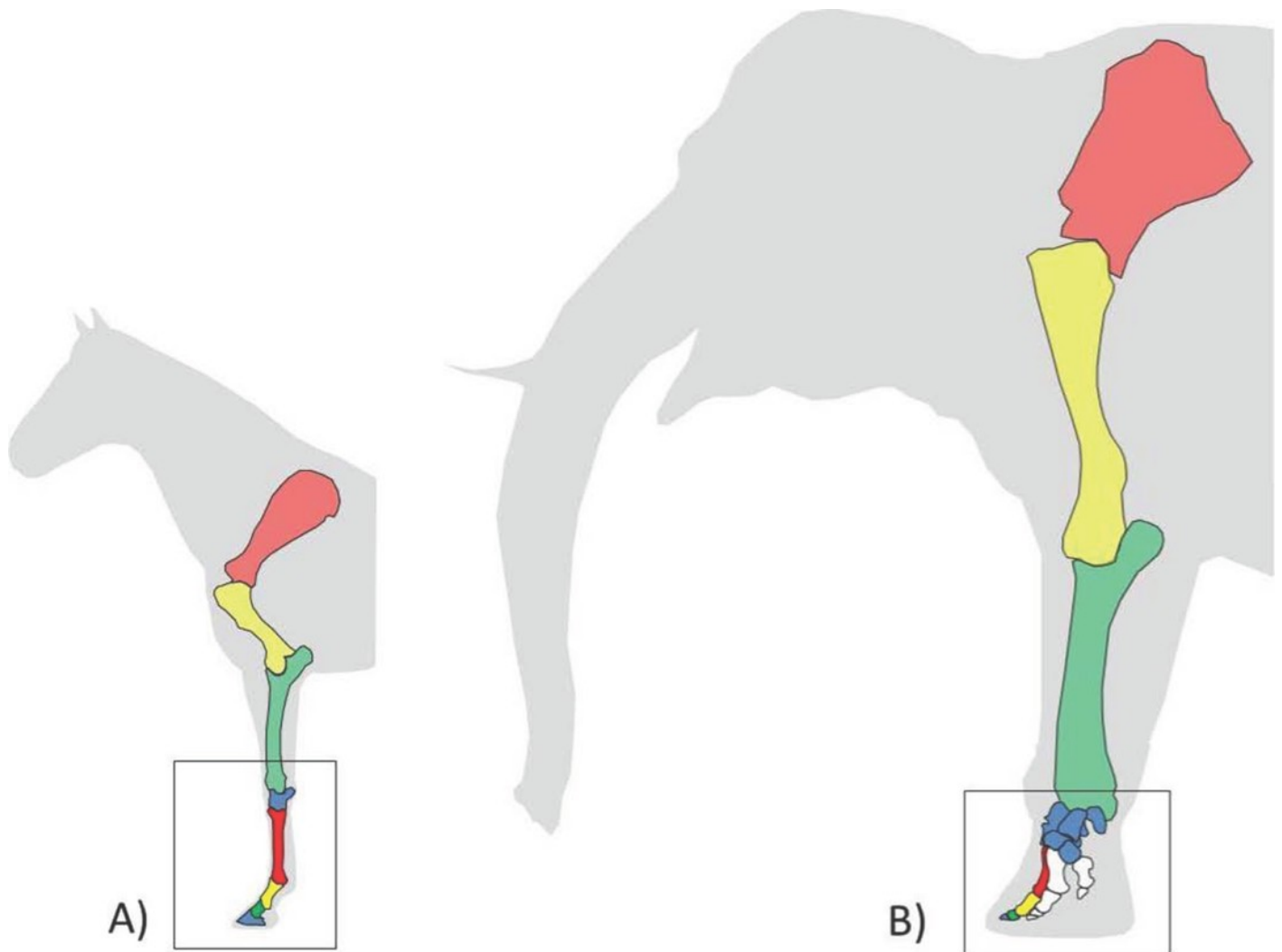


Figure 2

High resolution MR images of horse (A) and elephant (B) digital cushions (sagittal cross sections).

Digital cushions are outlined in red; compartments are outlined in white. No compartments can be visually detected in horse digital cushions, whereas it appears the smaller (micro) chambers are located more superficially than the larger (macro) chambers in elephant digital cushions.

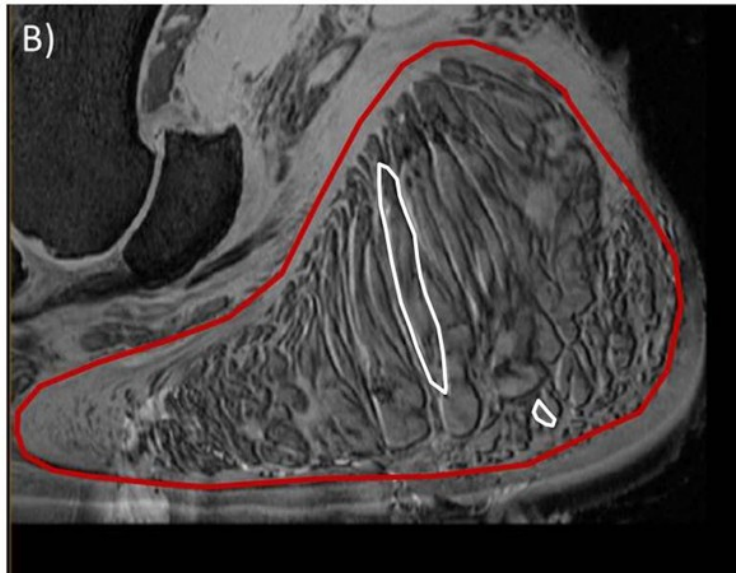


Figure 3

Internal pressure sampling (needle) locations in horse digital cushions.

A) Sagittal MR section showing intended sampling locations within the digital cushion (pink), Red shows the deep central location, blue shows the superficial central, yellow shows the superficial medial and green shows the superficial lateral location. B) Lateral radiograph with needles *in situ*. C) coronal MR section showing intended sampling locations within the digital cushion. D) dorsal-palmar radiograph with needles *in situ*.

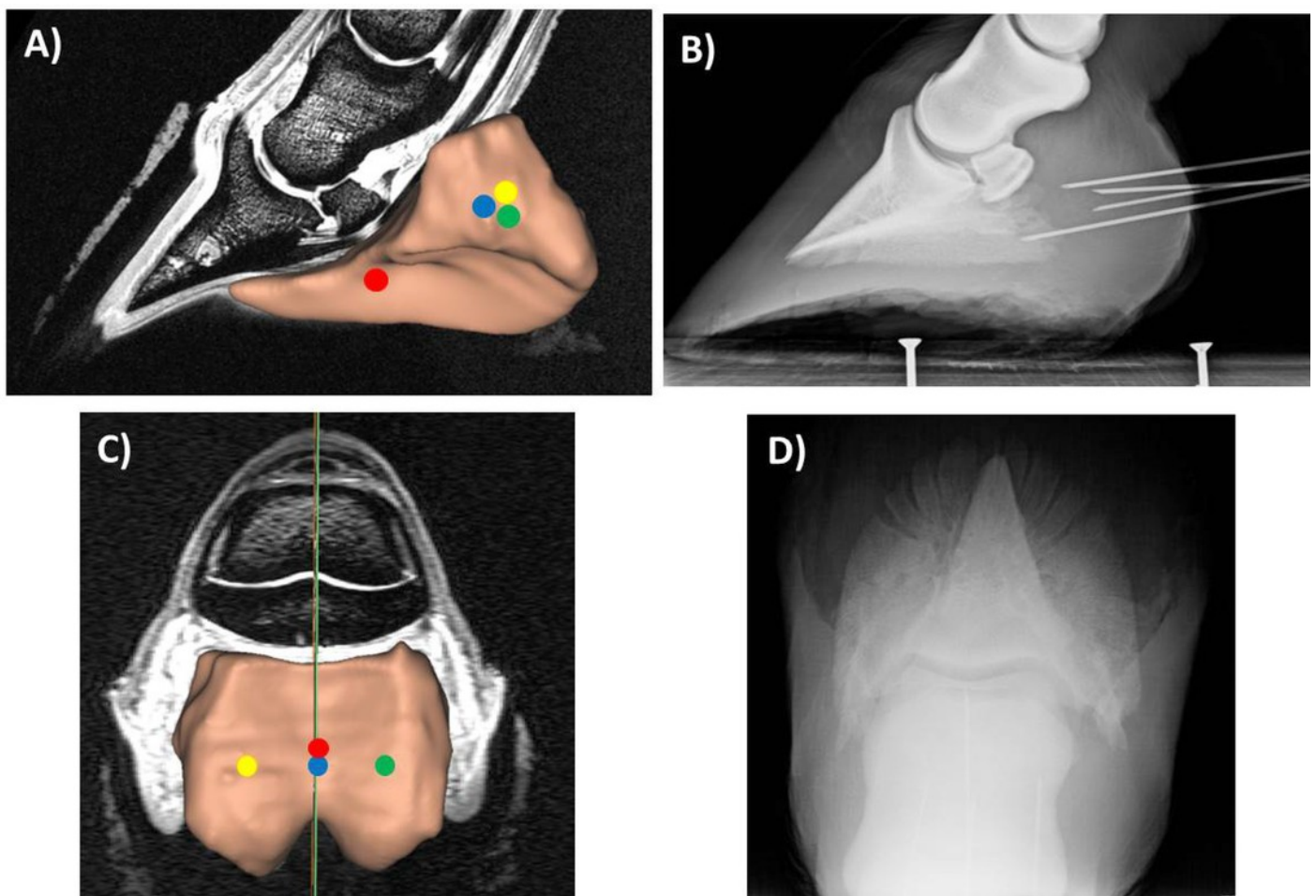


Figure 4

Internal pressure sampling (needle) locations in elephant digital cushions.

A) Sagittal MR section showing intended sampling locations within the digital cushion (pink), Red shows the deep central location, blue shows the superficial central, yellow shows the superficial medial and green shows the superficial lateral location. B) Sagittal CT section with needles *in situ* (only the deep central needle is visible). C) coronal MR section showing intended sampling locations within the digital cushion. D) Axial CT section with needles *in situ* (needle tips are shown in turquoise).

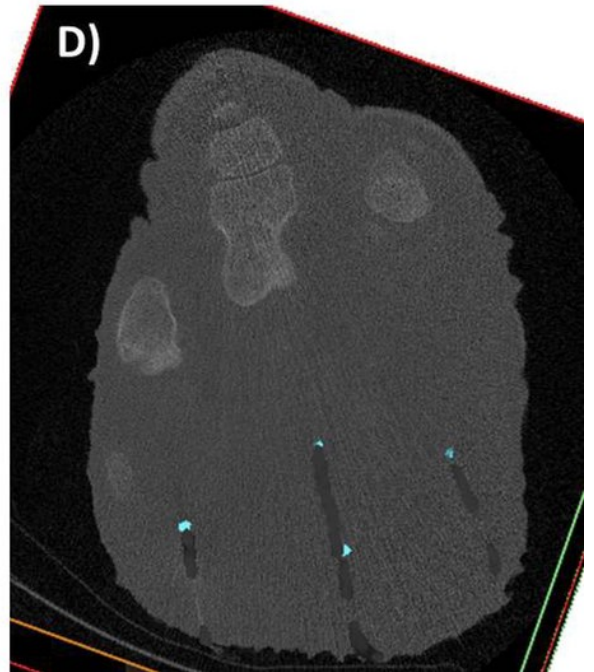
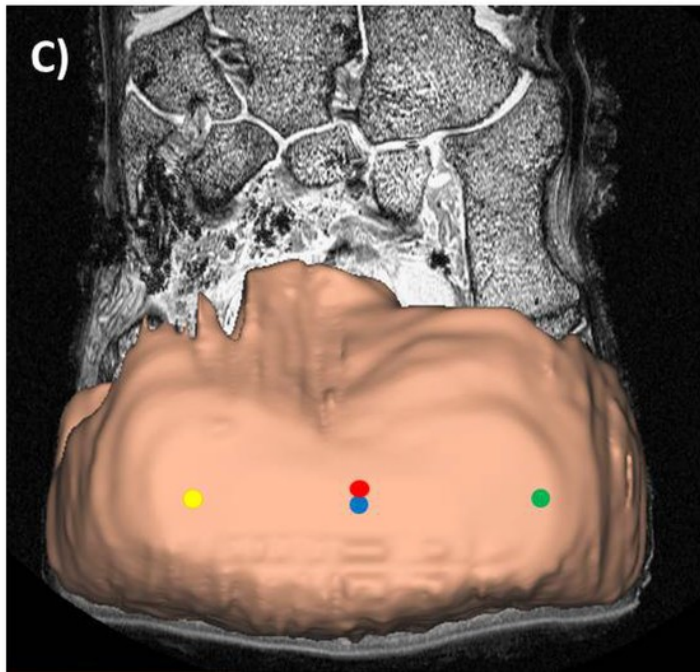
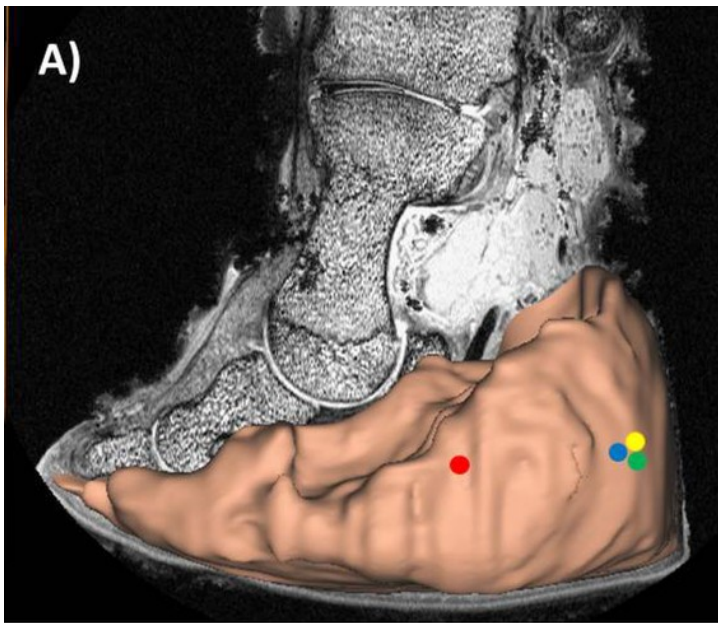


Figure 5

Data output from a 12 minute trial (horse limb loaded to 112 % body weight).

A) Pressure (mmHg) from the four transducers measuring internal digital cushion (DC) pressure and B) applied force (kN) from the Kistler force plate. Data were averaged over a 30 second window (grey shaded areas); the change in internal pressure was calculated by subtracting the average pressure under no load, from the average pressure under load to give mean internal pressure as a result of load. Maximum instantaneous pressure was also recorded (i.e., the maximum pressure change following load application) as was the rate of force application.

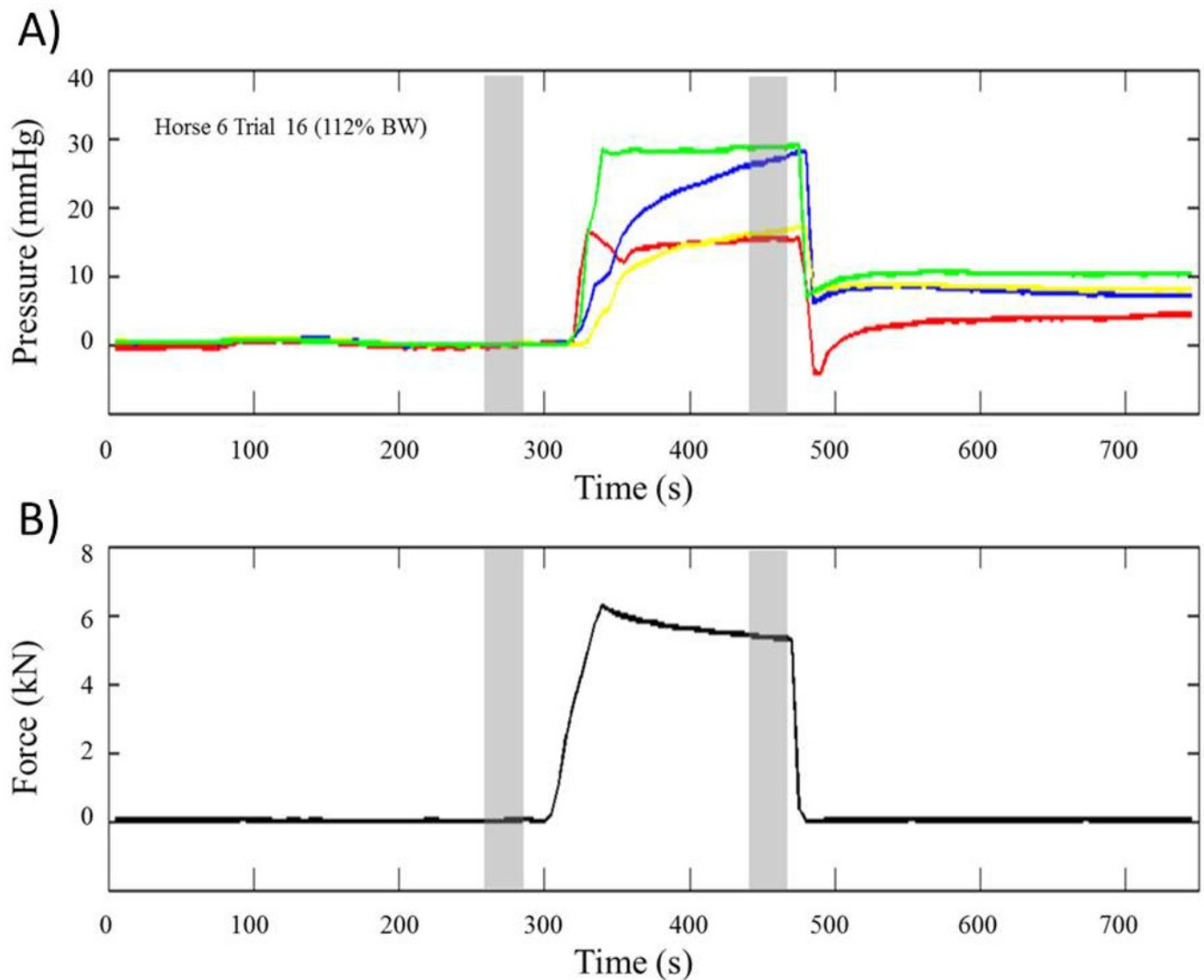


Figure 6

Method to determine x,y location of needle tips within the horse digital cushion.

Using dorso-palmar (A) and medio-lateral radiographs (B), the needle tips could be expressed as a percentage of foot width (x) and length (y). This information (i.e., the coordinates) was transposed onto the external pressure maps (C and D), to identify the location of the nine pressure pad sensors closest to the x,y needle tip. External pressures were calculated by averaging pressure across these nine sensors. Warm colours in C denote higher pressures, whereas cool colours denote lower pressures, black areas show zero pressure. Red is for the deep central, blue is for the superficial central, yellow is for the superficial medial and green is for the superficial lateral needle location, shown by the colour coded crosses.

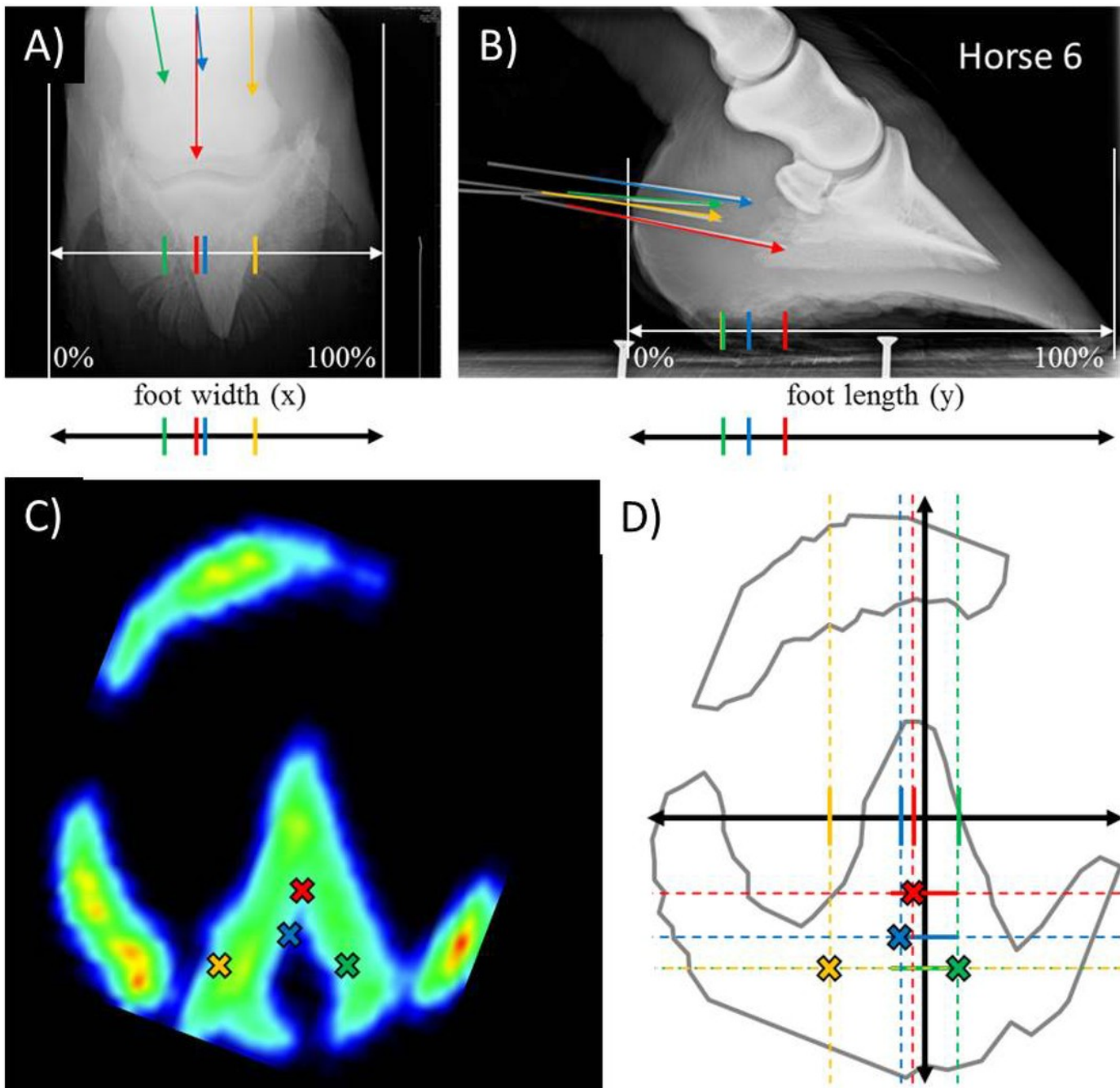


Figure 7

Method to determine x,y location of needle tips within the elephant digital cushion. <!--? R❖

Using reconstructed three dimensional (3D) models from computer tomography (CT) data (A and B), the location of each needle tip could be expressed as a percentage of foot width (x) and length (y). This information was transposed onto the external pressure maps (C and D), to identify the location of the nine pressure pad sensors closest to the x,y needle tip. External pressures were calculated by averaging pressure across these nine sensors. Warm colours in C denote higher pressures, whereas cool colours denote lower pressures, black areas show zero pressure. Red is for the deep central, blue is for the superficial central, yellow is for the superficial medial and green is for the superficial lateral needle location, shown by the colour coded crosses.

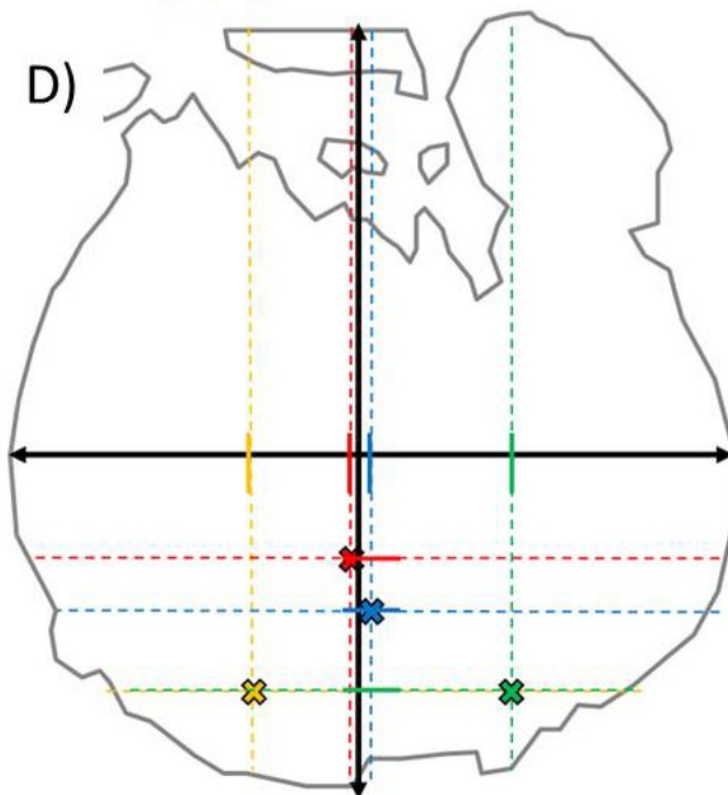
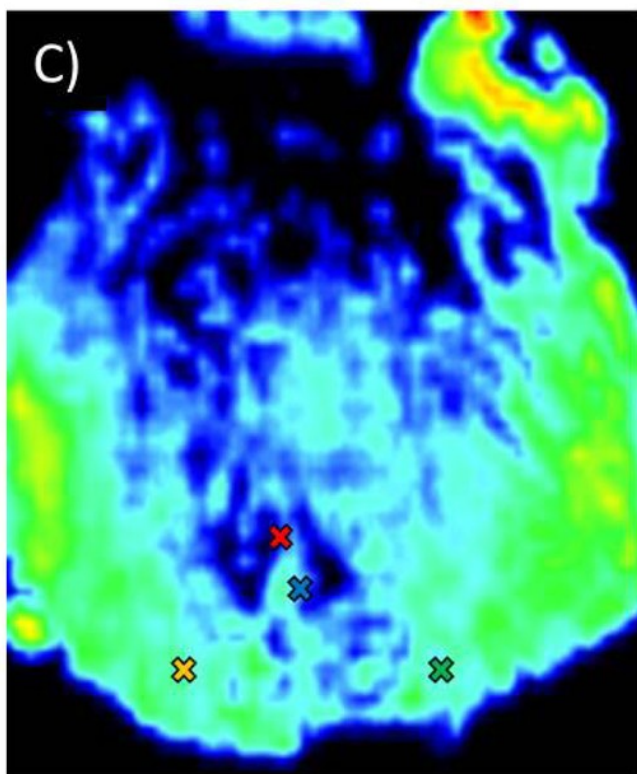
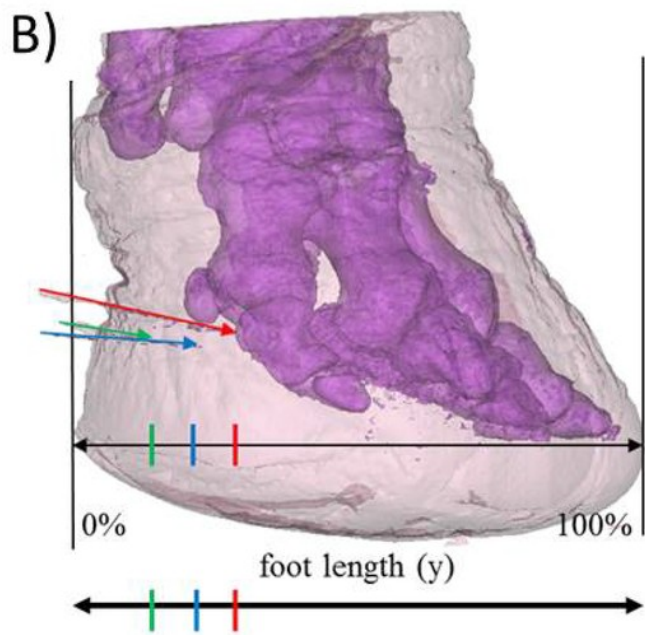
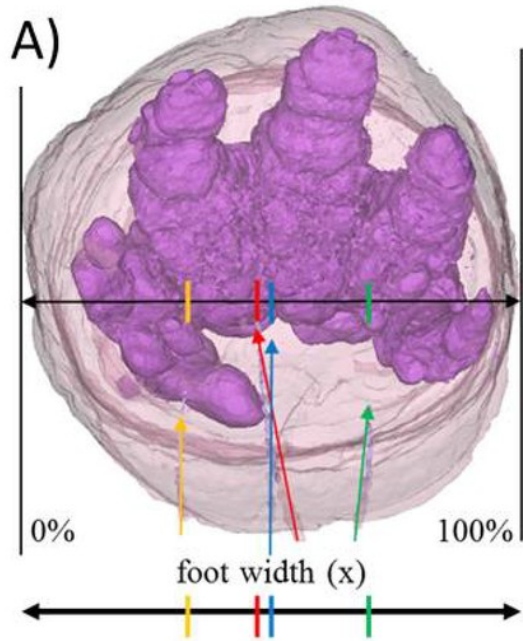


Figure 8

Radiographs showing variation in (horse) foot conformation.

Needle placement was difficult to replicate across individuals based on external foot landmarks.

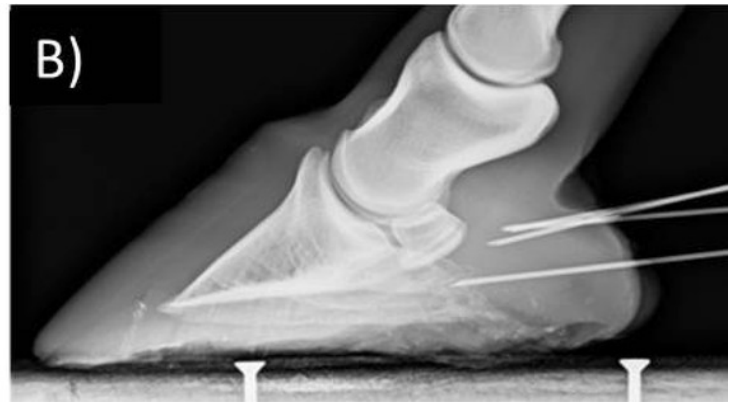


Figure 9

Normalised needle tip locations (as a % of foot width, foot length and proximal or distal phalanx height).

A) Lateral view of horse foot, B) lateral view of an elephant foot, C) palmar view of horse foot, D) palmar view of an elephant foot. Colour coded filled circles show where pressures in each region were sampled; red is for deep central, blue is for superficial central, yellow is for superficial medial and green is for superficial lateral pressures.

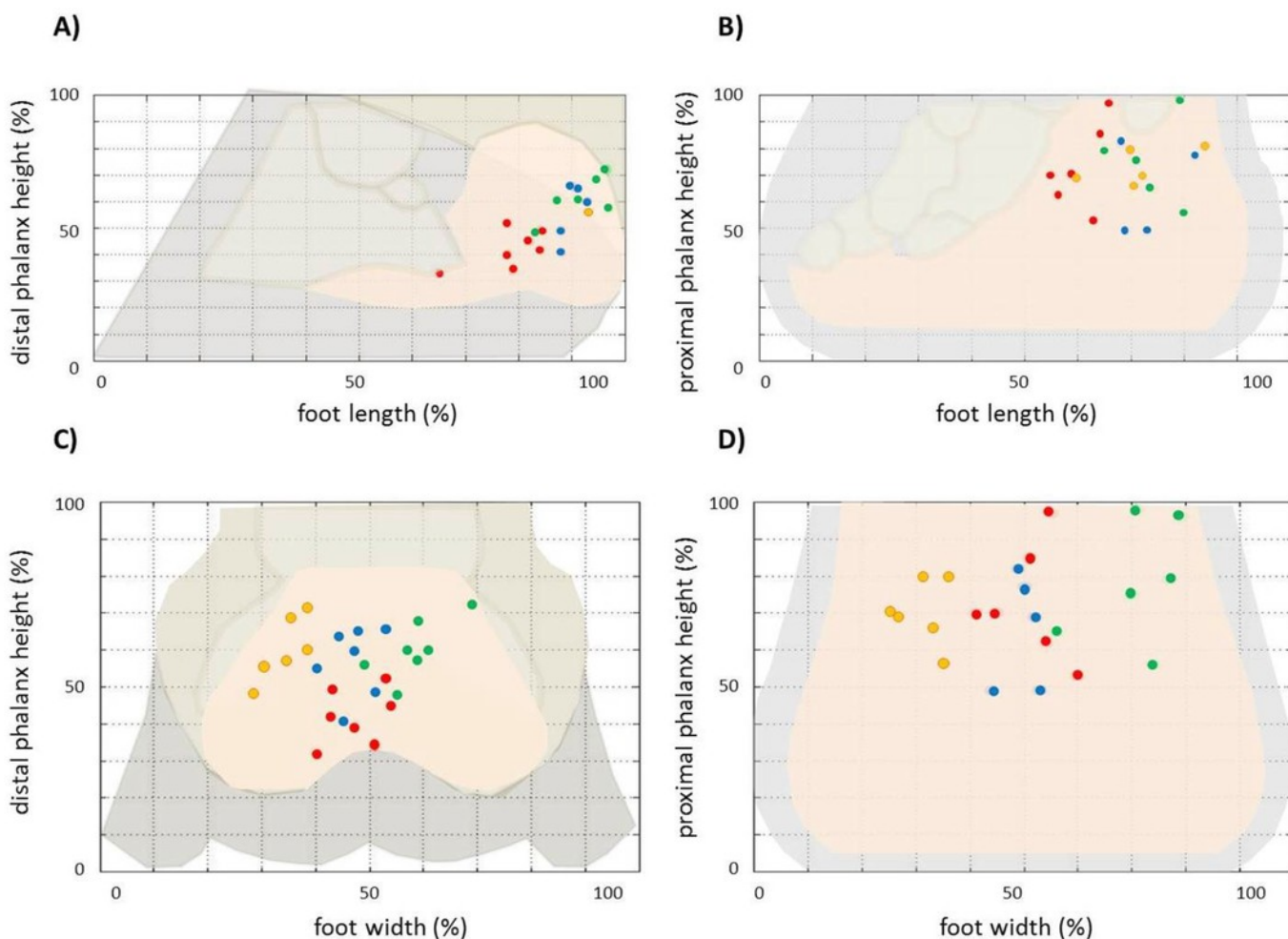


Figure 10

LMER Results: Mean internal pressure in each digital cushion (DC) location for A) horses and B) elephants.

Mean internal pressure (Nm^{-2}) is plotted against the applied force (N, from the force platform). Colour coded markers show pressure magnitudes in each region; red circles are for deep central, blue triangles are for superficial central, yellow diagonal crosses are for superficial medial and green vertical crosses are for superficial lateral pressures. N.B. the x axis is different between plots, 0-7000 N in horses represents loads of 0-150 % body weight (BW) (A), whereas 0-30,000 N in elephants represents loads of 0-60 % BW (B). Applying larger loads would have exceeded the force platform's maximal capacity.

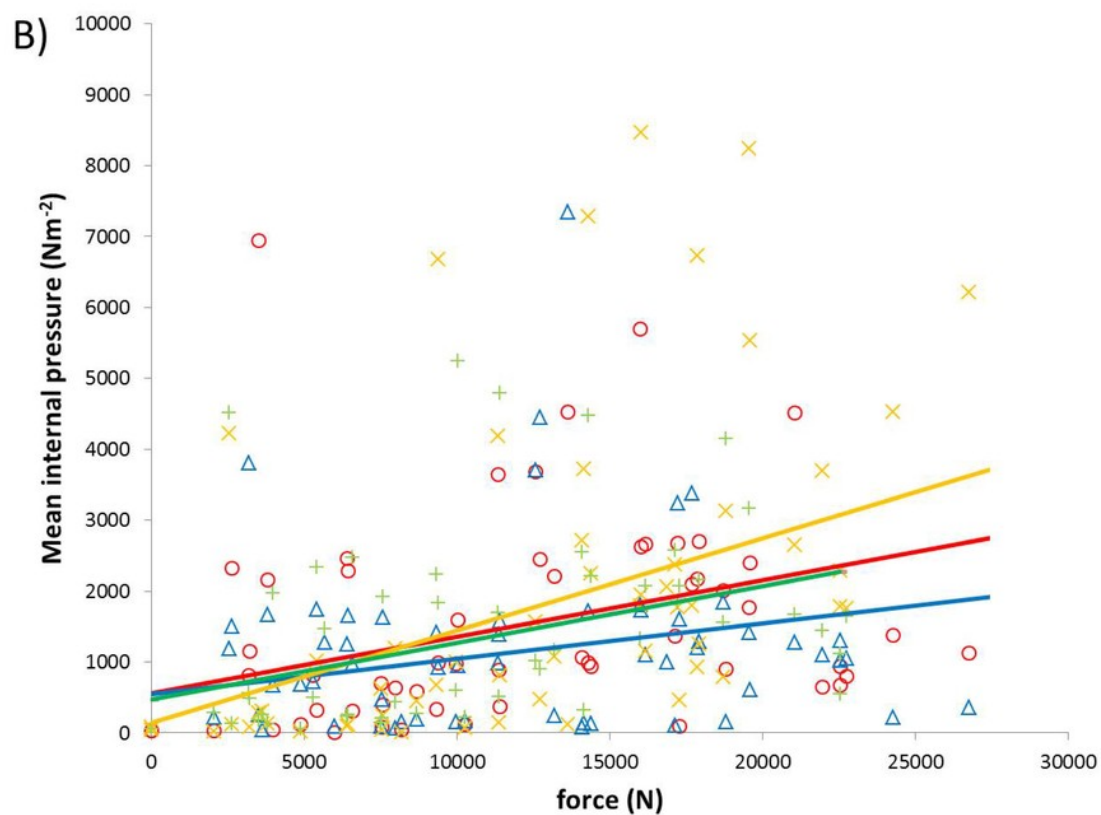
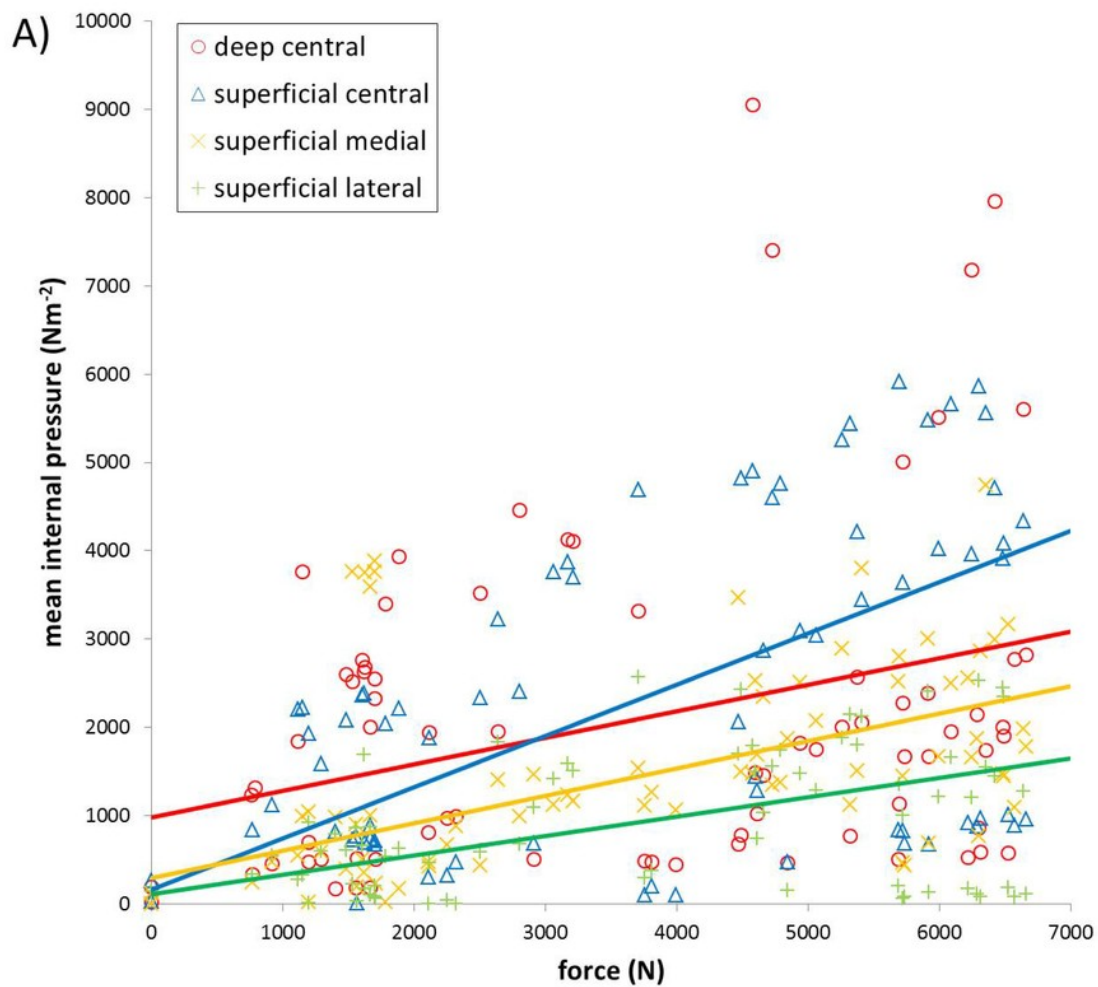


Figure 11

Species comparison: mean internal pressure in each digital cushion (DC) region.

A) Deep central, B) superficial central, C) superficial medial, D) superficial lateral. Triangles with the solid (LMER) line denote horse data, circles with the dashed (LMER) line denotes elephant data. P values show whether the slopes of the lines are significantly different between taxa.

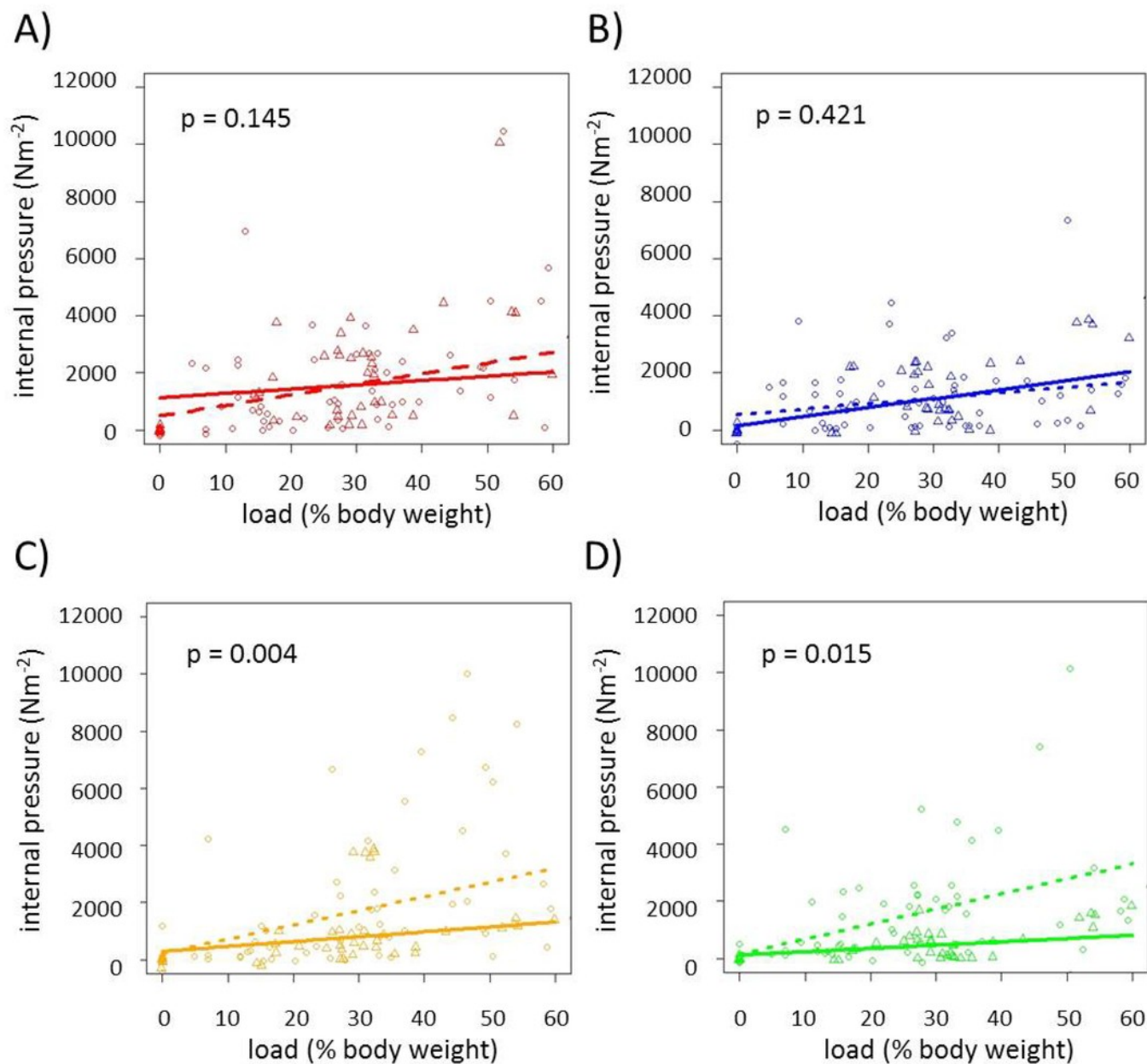


Table 1 (on next page)

Subject information.

Missing information is unknown. Details of the gross pathologies evident in elephant limbs are in Data S1. Asterisks (*) denote that the value is estimated (e.g. Miller *et al.*, 2008, or by post-mortem staff, based on relative stature of the horse).

subject	limb	breed	age	shoulder height (cm)	body weight (kg)	foot circumference (cm)	surface area (cm ²)	gross pathology evident
Elephant 1	RF	Asian		293*	3000*	130	1202	yes
Elephant 2	RF	Asian	17	293	3000*	133	1252	no
Elephant 3	RF	Asian	55	386*	3960	142	1401	yes
Elephant 4	LF	African		244*	2500*	112	908	yes
Elephant 5	RF	African	32	300*	3500*	132	1222	no
Elephant 6	LF	Asian	39	290	5500	140	1378	no
Horse 1	RF	mixed	19	122*	700*	44	131	no
Horse 2	RF	mixed	20	78*	450*	50	169	no
Horse 3	RF	mixed	25	78*	450*	47	137	no
Horse 4	LF	mixed		96*	550	47	160	no
Horse 5	LF	mixed		113	650	57	207	no
Horse 6	LF	mixed	5	109	536	50	180	no
Horse 7	RF	mixed	4	105*	602	53	197	no

Table 2 (on next page)

Results of the LMER analysis (see Methods).

Mean and maximum instantaneous internal pressure in both species. $y = mx + c$, where y is pressure (Nm^{-2}) and x is force (N). * denotes the values is significantly different from zero, p values for the slope (m) are shown.

	Mean Internal Pressure						Max. Inst. Internal Pressure					
	Horse			Elephant			Horse			Elephant		
	intercept	slope	p	intercept	slope	p	intercept	slope	p	intercept	slope	p
deep central (d.c.)	980*	0.30*	0.004	556	0.08*	0.008	1334*	0.28*	0.004	943	0.09	0.176
sup. central (s.c.)	164	0.58*	< 0.001	547	0.05*	0.048	255	0.59*	< 0.001	495	0.10*	0.016
sup. medial (s.m.)	113	0.22*	< 0.001	144	0.13*	0.002	72	0.28*	< 0.001	-91	0.29*	0.007
sup. lateral (s.l.)	293	0.31*	< 0.001	474	0.08*	0.002	384	0.32*	< 0.001	481	0.10*	0.002

Table 3(on next page)

Mean internal pressure.

Comparing linear coefficients from the LMER analysis for each DC location. Pressures that are significantly different are emphasised in bold.

	Horse				Elephant			
	d.c.	s.c.	s.m.	s.l.	d.c.	s.c.	s.m.	s.l.
deep central (d.c.)		0.718	<0.001	<0.001		0.161	0.002	0.183
sup. central (s.c.)	0.718		<0.001	<0.001	0.161		<0.001	0.107
sup. medial (s.m.)	<0.001	<0.001		0.001	0.002	<0.001		0.011
sup. lateral (s.l.)	<0.001	<0.001	0.001		0.760	0.107	0.011	

Table 4(on next page)

Maximum instantaneous internal pressure.

Comparing linear coefficients from the LMER analysis for each DC location. Pressures that are significantly different are emphasised in bold.

	Horse				Elephant			
	d.c.	s.c.	s.m.	s.l.	d.c.	s.c.	s.m.	s.l.
deep central (d.c.)		0.778	<0.001	<0.001		0.611	<0.001	0.866
sup. central (s.c.)	0.778		<0.001	<0.001	0.611		<0.001	0.520
sup. medial (s.m.)	<0.001	<0.001		0.126	<0.001	<0.001		<0.001
sup. lateral (s.l.)	<0.001	<0.001	0.126		0.866	0.520	<0.001	

Table 5(on next page)

Previously reported external palmar foot pressures.

Mean values are shown during walking (N.B. speed was not controlled in these studies).

	Horse	Elephant
max <i>in vivo</i> pressure (kNm ⁻²)	460.4 ^b	62.5 ^a
predicted peak palmar pressure (kNm ⁻²) (using M ^{0.5})	237.3 ^c	594.3 ^c

All values are means.

^aPanagiotopoulou *et al.*, 2012; ^bOosterlink *et al.*, 2011; ^cMichilsens *et al.*, 2009.

Table 6(on next page)

Results for external palmar pressures.

Palmar surface area was determined manually using foot circumference, and contact surface area was calculated from the number of sensors with values $> 0 \text{ Ncm}^{-2}$ from the pressure pad. The ratio of foot cross sectional areas (foot CSA): digital cushion cross sectional area (DC CSA) was calculated from axial CT data at the level of the needles. All values are median (\pm IQR).

	Horse	Elephant
Palmar surface area (m ²)	0.017 (0.004)	0.124 (0.014)
Contact surface area (m ²)	0.005 (0.003)	0.056 (0.036)
Ratio palmar surface area : contact surface area	3.38 (2.18)	2.34 (1.83)
Ratio foot CSA: DC CSA	5.09 (1.09)	3.09 (1.08)
Mean <i>ex vivo</i> external pressures ~30% BW (kNm ⁻²)	7.2 (6.6)	4.3 (1.1)
Max. <i>ex vivo</i> external pressures ~30% BW (kNm ⁻²)	63.0 (23.0)	36.0 (102.0)