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Asymmetries of forelimb digits of young cattle

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Abstract: Based the anatomical premise that in bovines, the medial (inner) claw is larger than the lateral (outer) one in the forelimb, we hypothesized that it implies a phalangeal form difference. To test this hypothesis, we studied asymmetries of the forelimb acropodia (phalangeal series) on calves belonging to Brown Pyrenean breed, a meat breed managed under extensive conditions in NE Spain. Dorso-palmar radiographies were obtained for each acropodium and form (size+shape) in a sample of 34 forelimbs (17 right and 17 left) from 17 animals. Images were studied by means of geometric morphometrics on medial and lateral acropodial series (III and IV series respectively) for each left and right forelimb. Shape coordinates were computed by Generalized Procrustes Analysis. Acropodial series resulted similar in size, but their shape expressed an especially high directional asymmetry, with distal phalanges (pedal bones) being abaxially oriented. Such morphological adjustments may be an important reconsideration of "normal" radiographic acropodial evaluation.

Keywords: digital bones; directional asymmetry; laterality; locomotion; Pyrenean Brown

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Introduction

In bilaterally symmetric structures, size and shape can differ between sides [1] [2]. Bilateral asymmetries can be expressed as fluctuating asymmetry and as directional asymmetry [3] [4] [5]. Fluctuating asymmetry (FA) is generally the result of genetic or environmental stress [6] [7] [8] [9] and is considered a negative indicator of the ability to resist random and small developmental accidents [3] [10]. Directional asymmetry (DA) occurs when there is a consistent form difference between sides [11] [5] [12] and generally it has a genetic basis [13], usually not being considered to be due to environmental or developmental stress [12].

The cattle metacarpus is composed of the two totally fused third and fourth metacarpal bones [14] [15]. This composite bone, colloquially known as "cannon bone", provides two separate epiphyses for the third and fourth digits [14] [15]. Although cattle has four digits, the abaxial series (II and V) are vestigial and incomplete [14] [15] whereas axial series (III and IV) are composed of three primary (distal -the pedal bone-, middle, and proximal phalanxes, the acropodia) and three secondary (one navicular and two sesamoids) bones [16] [15].

Like most bone skeletal structures, digits are generally assumed to be bilaterally symmetrical. The lateral (outer, IV) *vs* medial (inner, III) symmetry of acropodial series corresponds to matching symmetry, e.g. III and IV separated series appear as mirror images of each other [17]. The object symmetry, in contrast, is referred to a single structure which is identical according to a given or selected plane, such as mid-sagittal one [17].

Use of traditional (linear) morphometrics in studies of bilateral asymmetry has some limitations [18]. For instance linear distances omit information about relationships involving the shape [18]. Geometric morphometrics (GM) is based on a set of Cartesian coordinates points (x,y) that are homologous across individuals [19]. In contrast to traditional linear methods, GM preserves the geometry of the measured landmark configurations, and statistical results can thus be represented as shape deformations [19] [20] [18]. Moreover, morphometric methods are very helpful in standardizing and separating the different sources of asymmetry [19] [21]. Analysis of asymmetries in appendicular (such as the autopodes) or in axial skeletal elements are very scarce in cattle [22] [23] [24] [25]. In cattle the surface of lateral and medial hooves is no equal [14] [26] but GM has never been applied to limb asymmetry research in bovines before. The aim of this study was to determine asymmetries in acropodial series in calves based on radiographs as to our knowledge, radiographic geometric morphometric analyses of digits have not previously been used for cattle. Study has been centered on forelimbs, which take on a lesser role in propulsion [14] and so less prone to change according to substrates. As the chosen sample belonged to animals not subjected to foot care it is expected to describe "natural" acropodial asymmetries, at least among young animals similar to studied breed.

Materials and methods

A sample of 34 forelimbs from 17 Pyrenean Brown abattoir calves (9-15 months and 212-282 kg carcass weight as standard values) [27] was selected and labelled in a commercial slaughterhouse (17 right forelimbs and 17 left forelimbs). The animals were selected randomly and were clinically sound according to *ante mortem* abattoir official veterinary inspection. None of them had been had previous trimming. Individual information was not possible for samples, therefore sex and carcass weight for each animal could not be considered, although there were no castrated animals. The limbs were identified as the feet came off the slaughter line. Each set of 2 paired feet was then placed in a uniquely identified plastic bag. The feet were brought back to the Department of Animal Science and stored in plastic bags at -18°C until processing.

Images were obtained using a computed radiographic system (Potro DR®) following standard procedures [28]. The exposure factor for dorso-palmar view were 60 kV and 3.2 mAs with each defrosted foot to be examined with the x-ray beam centered approximately to the metacarpophalangeal joint (Figure 1). The cassette was vertically positioned as close as possible to the metacarpus. A total of seven landmarks occurring on both sides (axial and abaxial) of the acropodial joints (6 landmarks), abaxial distal metacarpal epiphysis (1 landmark) and one axial on distal metacarpal epiphysis were used (Figure 1). These landmarks were chosen in order to have a good representation of the overall acropodial shape and to detect important features of asymmetry. The captured images were transformed to TpsUtil v. 1.40 software [29] and landmarks recorded using TpsDig v. 2.26 software [30].

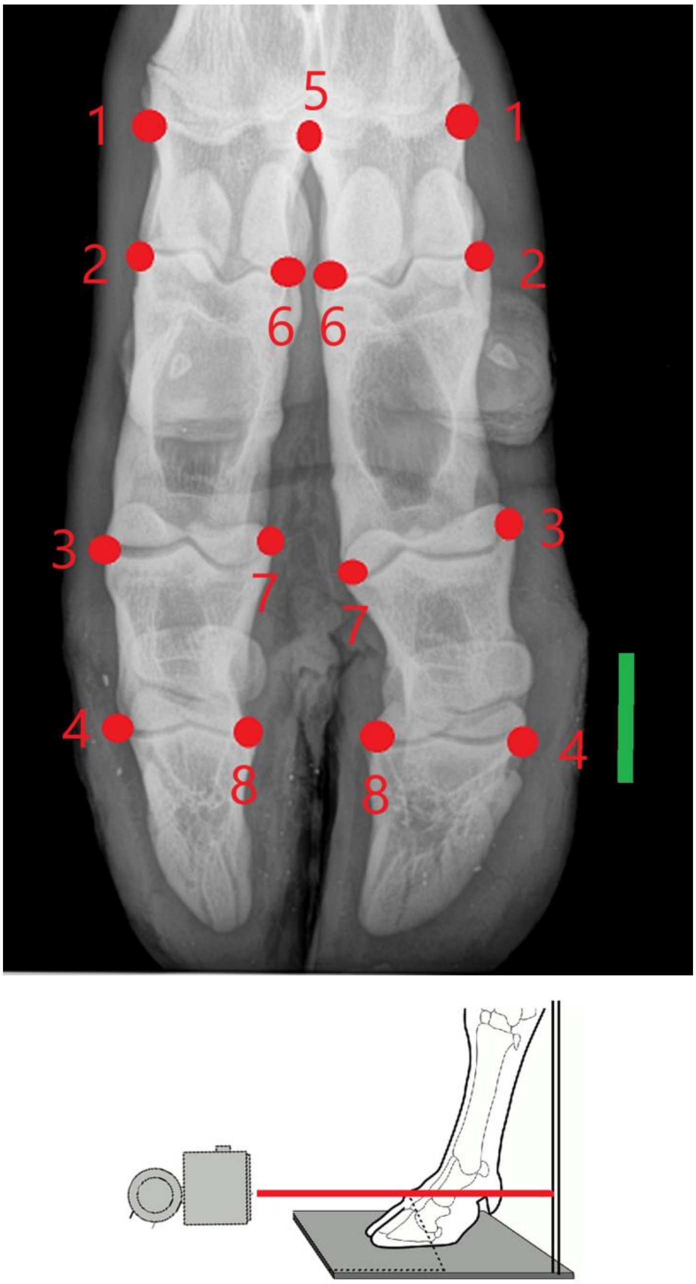


Figure 1. Dorso-palmar view of acropodium on which seven landmarks occurring on both sides of the acropodiums joints (2 to 4, and 6 to 8), disto-abaxial metacarpal epiphyseal bands (1) and one axial (no. 5 on the distal metacarpal epiphysis) were located. Bar is 3 cm long. For each limb the x-ray beam centered approximately to the metacarpophalangeal joint.

To obtain the shape data, landmark configurations were superimposed using the generalized Procrustes method, based on a generalized least-squares minimization of the distance between corresponding landmarks [31]. Landmark configurations were compared by this superimposition, which is achieved by translating, rotating and scaling all configurations to a common reference system (the mean) [21]. As a proxy for size we used the centroid size (CS), which corresponds to the squared root of the sum of the squared distances from each landmark to the centroid [31].

Statistical analyses

We used TpsSmall v. 1.33 software [32] to test whether the observed variation in shape was sufficiently small that the distribution of points in the tangent space could be used as a good approximation of their distribution in shape space [31]. Size differences were analyzed with an

ANOVA (ANalysis Of VAriance). Procrustes coordinates were assessed for asymmetry of shape in relation to individuals (symmetric component of variation), asymmetric component of variation (Directional Asymmetry DA and Fluctuating Asymmetry FA), and measurement error. FA was interpreted as the "individual*side" interaction effect, while DA as the "side" effect. This was done with a two-way NPMANOVA (Non Parametric Multivariate ANalysis Of VAriance), using "individuals" and "sides" as factors. Allometry was studied by means of a multivariate regression using CS (log-transformed) as independent variable and shape (expressed as Procrustes coordinates) as dependent variable. A subsequent shape analysis was done by a Principal Component Analysis (PCA) to simplify descriptions of differences between series. PCA was calculated from the var-covar matrix of the variables. Canonical Variate Analysis (CVA) was used to ordinate specimens to maximise separation of four series. We used regression scores for PCA and CVA. Finally, a representation of bending energy (thin plate spline) allowed to appreciate shape changes as forces acting on a coloured mesh that deforms according to the direction. The bending energy represents the idealized energy that would be required to bend the shape change plate so that the landmarks were lifted or lowered appropriately.

Analyses were done with MorphoJ v. 1.07a [33] and PAST v. 2.17c [34].

Results

First analyses indicated that an excellent correlation between the tangent and the shape space existed, as the correlation (uncentered) between the tangent space *Y* regressed onto Procrustes distance was 0.9995.

Size asymmetries

ANOVA reflected that III and IV acropodial series presented no size differences between them for both limbs ($F_{3,132}$=0.156; p=0.925).

Allometry

There appeared no significative regression of CS *versus* Procrustes coordinates ($F_{16, 119}$=0.719, R^2=0.0076, p=0.769), so there was no allometric effect of acropodial size *versus* its shape.

Shape asymmetries

Procrustes ANOVA for assessing measurement error showed that the mean square for individual variation (71.4%) exceeded the measurement error (6.1%), thus, the measurements were deemed reliable. There appeared directional asymmetries (DA, "side" effect) and fluctuating asymmetries (FA, interaction effect) for both series in each right and left forelimb. Net asymmetry (DA+FA) accounted for more than 90% of total variation, being DA more than twenty times bigger the value of FA (Table 1). In the PCA the first two principal components represented a 92.43% of total shape variability, with PC1 accounting for the most significant variance (76.17%) (Figure 2). There was a gradual decrease in percent variance with a drop between PC1 (76.17%) and PC2 (16.26%) (Figure 3) so PC2 and PC3 were ignored to describe shape features as they contributed little to the overall variation. The primary shape characteristic of the dataset as represented by the first PC may be interpreted as a horizontal displacement of distal landmarks -on distal phalanx- showed the highest contribution to variance (Figure 4). CV1 explained 98.6% of variation in the analyzed material and it clearly separated each group, with significative differences between them (Pillai's trace=2.67, $F_{48,357}$=60.17, p<<0.001). It was clear a shape closeness between left lateral-right medial, and right lateral-left medial series (Figure 5).

Table 1. Procrustes ANOVA for shape of right and left forelimbs of Pyrenean Brown calves (n=34), with a significant effect of "Side" (directional asymmetry) and interaction effect (fluctuating asymmetry) (9,999 permutation rounds). Sums of squares (SS) and mean squares (MS) are in units of Procrustes distances (dimensionless). Measurement error showed that MS for individual variation

exceeded the measurement error. There appeared directional asymmetries and fluctuating asymmetries, for both right (n=17) and left (n=17) forelimbs. Sums of squares and Mean squares are in units of Procrustes distances (dimensionless).

1/ Right forelimb (n=17)

Source	Sum of squares	Df	Mean square	*F*	*p*
Individual	0.032029	16	0.0020018	32.306	0.0001
Side	0.027964	1	0.0279640	45.128	0.0001
Interaction	0.018533	16	0.0011583	18.693	0.0002
Residual	0.021068	34	0.0006196		
Total	0.099594	67			

2/ Left forelimb (n=17)

Source	Sum of squares	Df	Mean square	*F*	*p*
Individual	0.033277	16	0.0020798	40.677	0.0001
Side	0.028572	1	0.0285720	55.881	0.0001
Interaction	0.019052	16	0.0011908	23.289	0.0001
Residual	0.017384	34	0.0005113		
Total	0.098285	67			

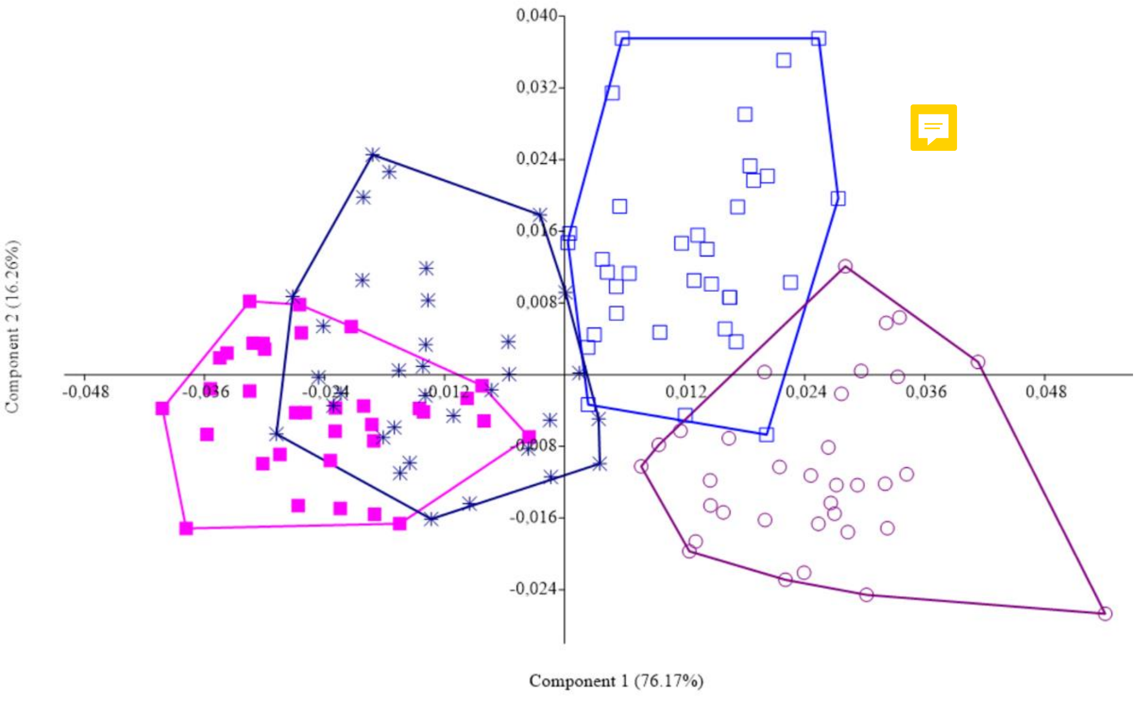


Figure 2. Principal Component Analysis for left and right forelimbs belonging to 17 Pyrenean Brown calves. The first two PCs were statistically meaningful and portrayed a 92.43% of total shape variability, with PC1 accounting for the most significant variance (76.17%). Empty dots: right medial acropodial series; empty squares: left lateral acropodial series; stars: right lateral acropodial series; filled squares: left medial acropodial series.

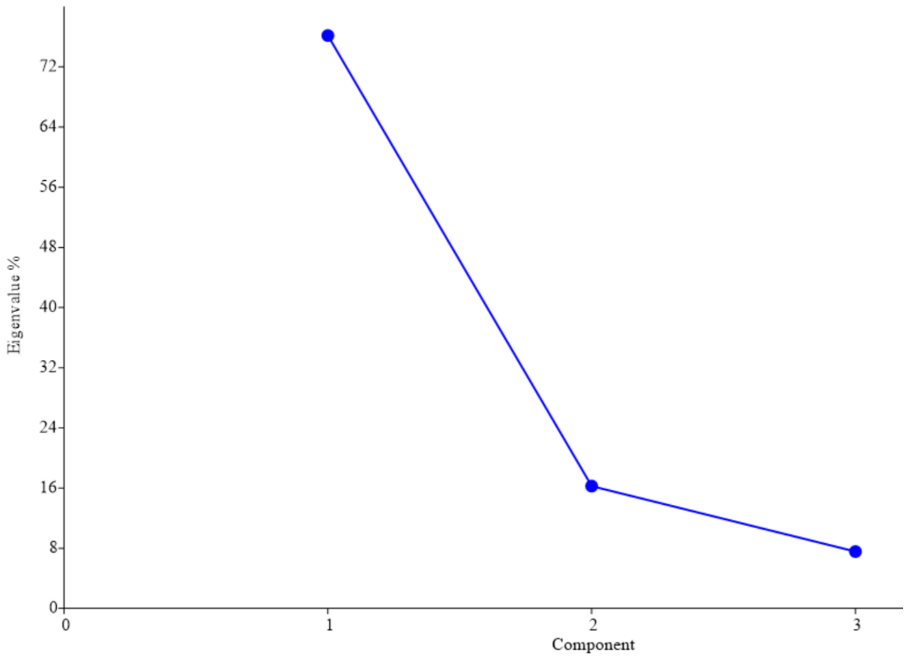


Figure 3. Screen plot showing the proportion of the total shape variance explained by each PC for 34 distal limbs belonging to 17 Pyrenean Brown calves. There was a gradual decrease in percent variance with a drop between PC1 and PC2. Note: the first three shown PCs accounted for all variation.

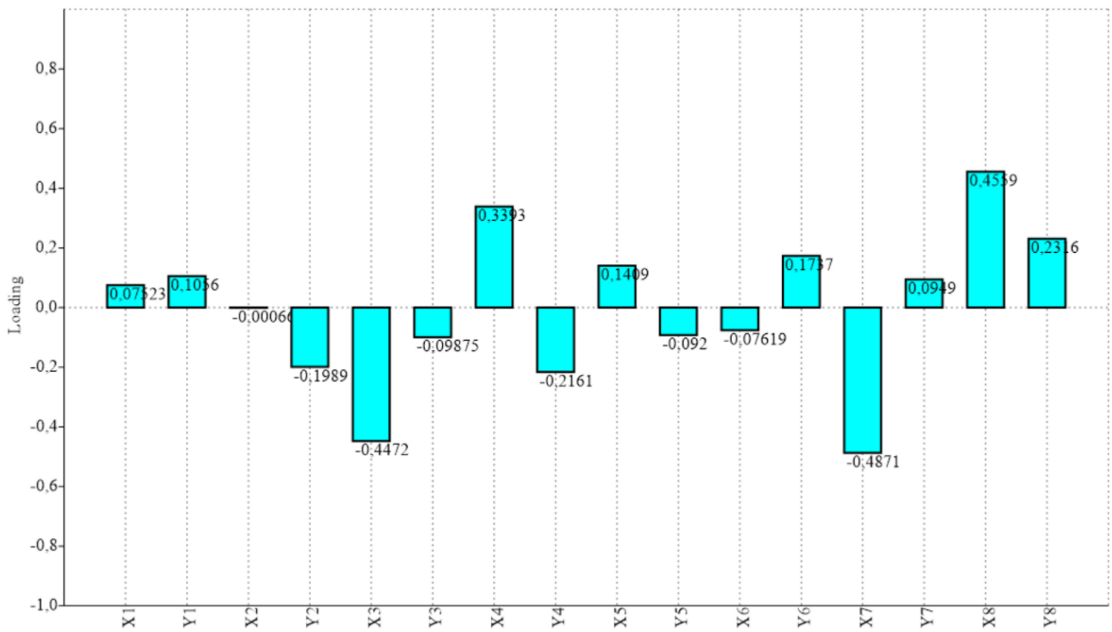


Figure 4. Loadings for the first PC which accounted for a 76.17% of the total observed variance for each of the 8 studied landmarks (expressed in x, y Cartesian coordinates).

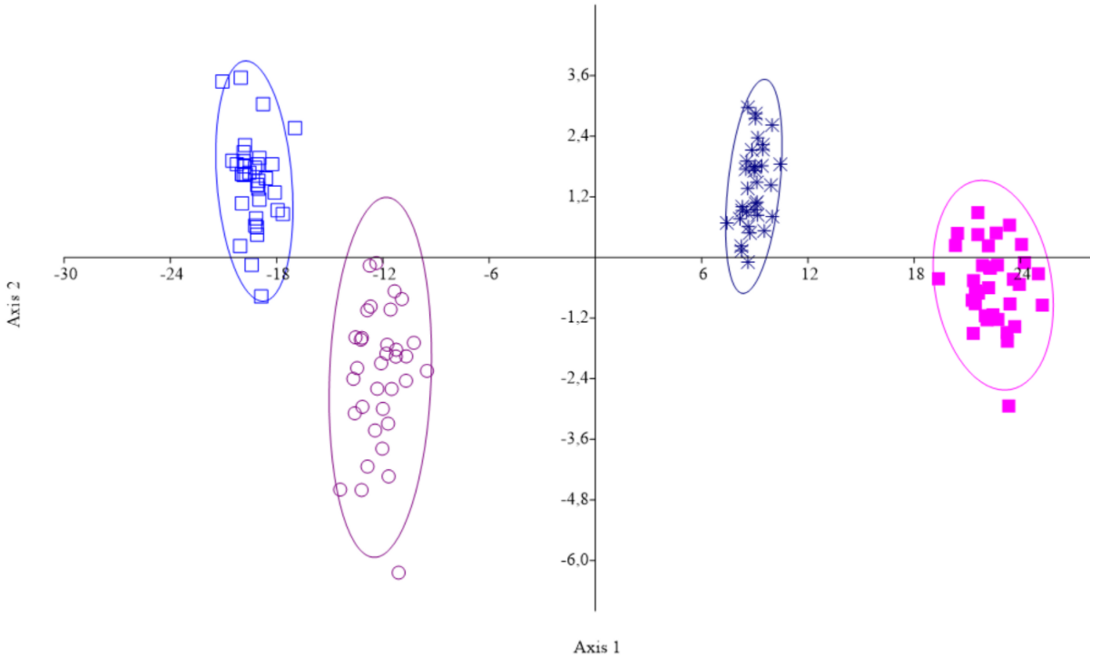
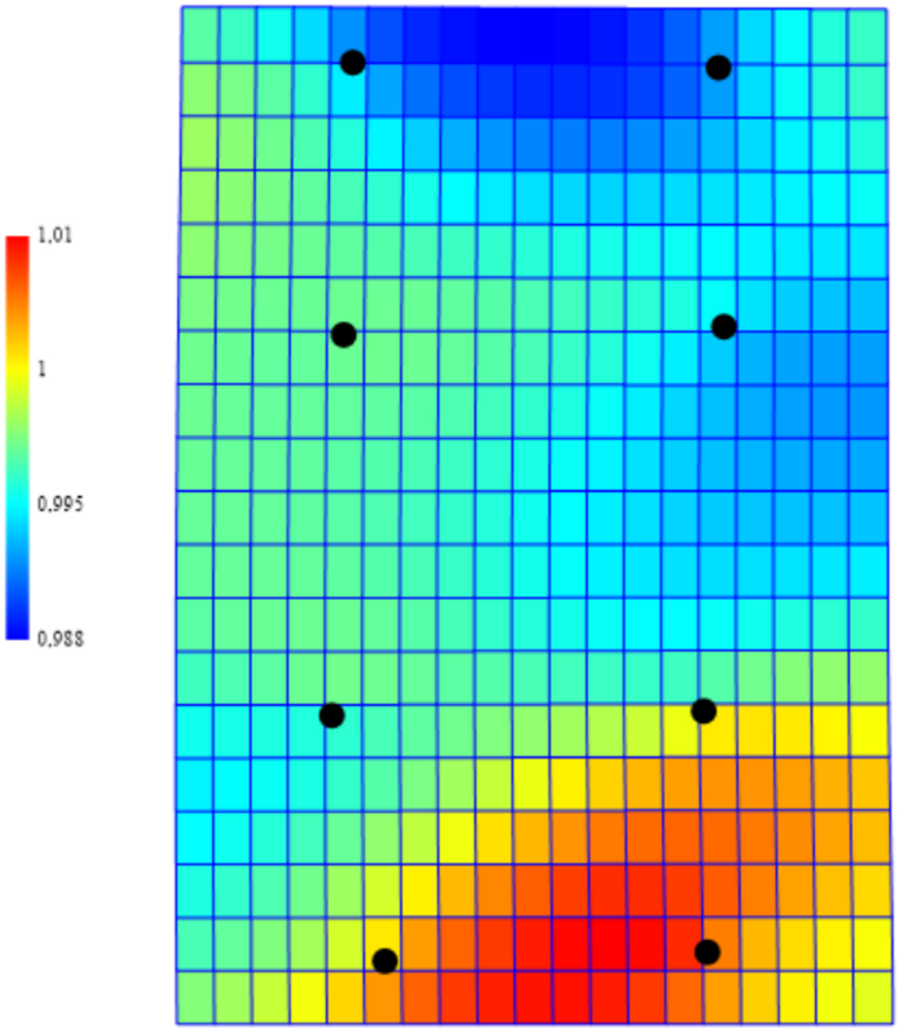
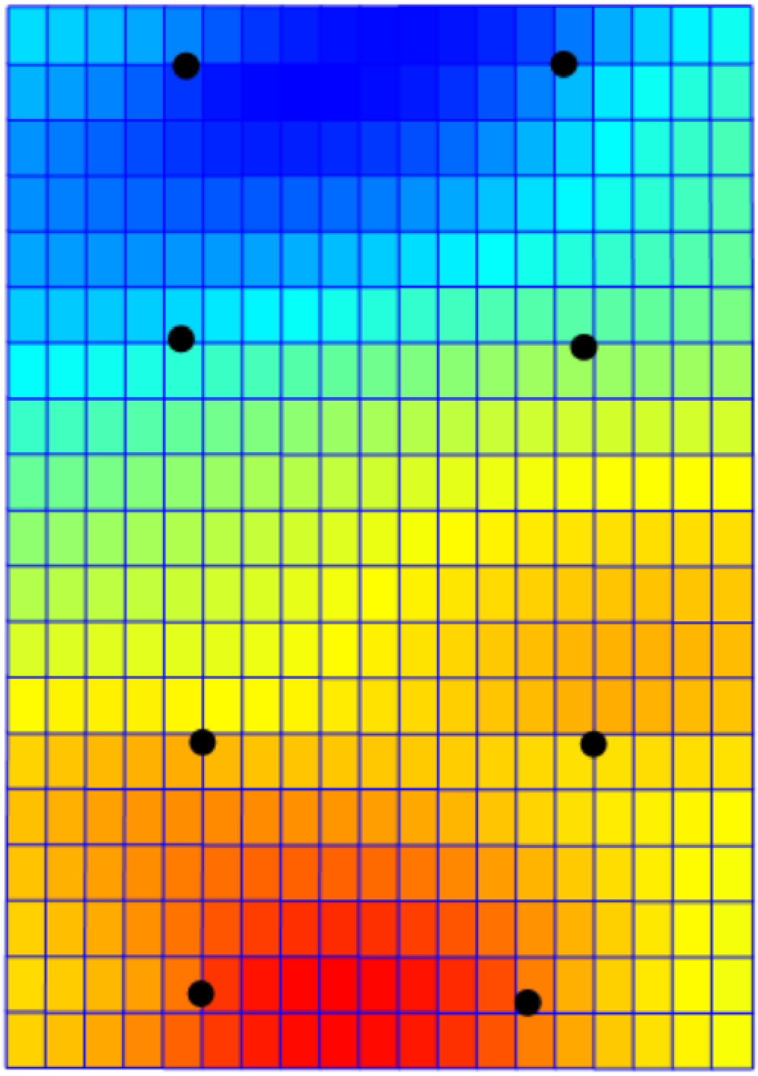


Figure 5. Canonical Variate Analysis of asymmetric shapes for left and right forelimbs belonging to 17 paired limbs from 17 Pyrenean Brown calves. Acropodial shapes appear plotted in two-dimensional space formed by the scores of the first two Canonical Variate. CV 1 explained 98.6% of the variance. Empty dots: right medial acropodial series; empty squares: left lateral acropodial series; stars: right lateral acropodial series; filled squares: left medial acropodial series.

In the representation of bending energy (contraction, bluish coloration and expansion, reddish coloration) it was observed a left (axially) displacement of distal phalanges in left lateral and right medial series, while this displacement was towards right (axially, too) for right lateral and left medial series. (Figure 6). In other words, and abaxial displacement for paired pedal bones.



1/ Left lateral and right medial series



2/ Left medial and right lateral series

Figure 6. Representation of bending energy (contraction, bluish coloration and expansion, reddish coloration) for 17 paired limbs from 17 Pyrenean Brown calves (dorsal views). It was observed displacements of distal phalanges with a tendency towards abaxial displacement for paired acropodial series.

Discussion

In this study we analysed acropodial asymmetries in young domestic cattle using radiographic images by means of geometric morphometric techniques. It appeared an undeniable presence of shape asymmetry in the studied sample. In cattle it has been described the medial hoof being the larger hoof in the forelimb [14] [26]. But this is not reflected on bones, as acropodial sizes are similar between medial and lateral series.

But this asymmetry was present in shape. Once net asymmetry of the studied sample was decomposed into directional asymmetry and fluctuating asymmetry, it was found that most of the variation in asymmetry was explained by directional asymmetry alone. Directional asymmetry refers to non-pathological differences between sides of the body which result from lateralized behaviours and biomechanical pressures [18].

The directional asymmetry can be a product of genotype as well as of lateralization in the muscle load or other functional demands [35]. The most probable explanation for the observed average asymmetry in acropodial bones is that it results from a functional splay. The distal phalanx -pedal bone- is in indirect contact with the ground through the hoof [14] [36]. The abaxial displacement imbalance on two paired pedal bones load would directly modulate their external morphology. So, detected directional asymmetry can be largely attributable to differential mechanical loading due to digital splay. This is consistent to the main vertical axis supporting the body weight in limbs, where the phalanges would tend to fall distally in it, so being differently developed, e.g. different shaped [37]. Radiographically it can be seen as an abaxially oriented of medial and lateral apexes of the hoof [36] [38].

In summary, our results showed significant differences in the paired shape -but not size- of the medial and lateral acropodia with a clear abaxial-biased distal asymmetry, which had to be seen as an important "normal" feature of acropodial conformation, at least in the studied breed. Now it would be interesting to study if this asymmetry is expressed sexually dimorphism and its behaviour in more aged animals, and to detect if this uneveness coincides with both kinetic and kinematic asymmetrical locomotor differences.

Author contributions: PMPC designed the study and directed implementation and data collection. LCN and KJC performed radiographies and collected data. PMPC, LCM and KJC analysed the data and drafted the manuscript.

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Conflict of Interest: The authors declare that there are no competing interests regarding the publication of this paper. There were no funders in study design, data collection and analysis, decision to publish, or preparation of the manuscript. It was not required an animal ethics committee approval from the Ethics Committee as we used remains of an animals that were killed for commercial purposes totally unrelated to this study ("DECRET 214/1997, de 30 de juliol, pel qual es regula la utilització d'animals per a experimentació i per a altres finalitats científiques".

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