Kafkas Universitesi Veteriner Fakultesi Dergisi
ISSN: 1300-6045 e-ISSN: 1309-2251
Journal Home-Page: http://vetdergikafkas.org

Kafkas Univ Vet Fak Derg
27 (6): 795-802, 2021
DOI: 10.9775/kvfd.2021.26337

RESEARCH ARTICLE

Determination of Gender and Breed in Arabian Horses and Thoroughbred Horses Using Radiography of the Tarsal Region

Dilek OLGUN ERDIKMEN [1,a] Ozan GUNDEMIR [2,b] Gulsun PAZVANT [2,c] Mustafa Orhun DAYAN [3,d]
Sokol DURO [4,e] Hulya HARTOKA [5,f] William PÉREZ [6,g] Cagla PARKAN YARAMIS [7,h (*)]

[1] Department of Surgery, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, TR-34320 Avcılar/Istanbul - TURKEY
[2] Department of Anatomy, Faculty of Veterinary Medicine, Istanbul University-Cerrahpaşa, TR-34500 Büyükçekmece, Istanbul - TURKEY
[3] Department of Anatomy, Faculty of Veterinary Medicine, Selçuk University, TR-42003 Konya - TURKEY
[4] Faculty of Veterinary Medicine, Agricultural University of Tirana, Tirana, ALBANIA
[5] Jockey Club of Turkey, Istanbul Horse Hospital, TR-34144 Istanbul - TURKEY
[6] Unidad de Anatomía, Facultad de Veterinaria, Universidad de la República, Montevideo, URUGUAY
[7] Vocational School of Veterinary Medecine, Equine and Equine Training Programme, Istanbul University-Cerrahpaşa, TR-34320 Avcılar, Istanbul - TURKEY
ORCIDs: [a] 0000-0002-8190-8429; [b] 0000-0002-3637-8166; [c] 0000-0001-5986-3992; [d] 0000-0003-0368-4607; [e] 0000-0002-6075-7342
[f] 0000-0002-6666-1851; [g] 0000-0002-9647-4731; [h] 0000-0002-5048-9452

Article ID: KVFD-2021-26337 Received: 04.08.2021 Accepted: 04.11.2021 Published Online: 05.11.2021

Abstract

In this study, it was examined whether the radiographic measurements taken from the tarsal region, especially the calcaneus, can be used in sex determination. In addition, two different horse breeds were used and whether these values would cause any difference between the breed was examined. 213 race horses were used in this study. Radiographic images of the left tarsal joint of Arabian horses (30 females, 57 males) and Thoroughbred horses (57 females, 69 males) were taken from the lateromedial direction. The widest point of the trochlea was seen to be a determinant in sex determination of Arabian horses. In Thoroughbred horses, it was seen that the value of the calcaneus body was the most important factor in sex determination. As a result of the discriminant analysis, 71.26% of male and female Arabian horses and 66.67% of Thoroughbred horses, respectively were classified correctly. It was observed that 88.26% of the horses between the two breeds were classified correctly regardless of sex. In the correlation test between measurements, negative correlation value with age was seen in the talus measurement (correlation value: -0.139-099). In conclusion, radiological measurements of this clinically important region can be used as an alternative method for sex and breed discrimination.

Keywords: *Calcaneus, Discriminant analysis, Equine, Limbs, Radiometric, Talus*

Arap ve İngiliz Atlarında Tarsal Bölgenin Radyografisi Kullanılarak Irk ve Cinsiyetin Belirlenmesi

Öz

Bu çalışmada başta calcaneus olmak üzere, tarsal bölgeden alınan radyografik ölçümlerin cinsiyet tayininde kullanılıp kullanılamayacağı incelenmiştir. Ayrıca farklı iki at ırkı kullanılmış ve alınan bu değerlerin ırklar arasında herhangi bir farklılığa neden olup olmadığı incelenmiştir. Çalışmada 213 yarış atı kullanıldı. Safkan Arap (30 dişi, 57 erkek) ve İngiliz atlarının (57 dişi, 69 erkek) sol tarsal eklemlerinin lateromedial yönden radyografik görüntüleri alındı. Arap atlarının cinsiyet tayininde trochlea'nın en geniş noktasının belirleyici olduğu görüldü. İngiliz atlarında ise cinsiyet tayininde kalkaneus gövde ölçüm değerinin en önemli faktör olduğu görüldü. Diskriminant analizi sonucunda cinsiyet ayırımında erkek Arap atlarının %71.26'sının, İngiliz atlarının ise %66.67'sinin doğru olarak sınıflandırıldığı görüldü. İki ırk arasındaki atların %88.26'sının cinsiyet gözetmeksizin doğru sınıflandırıldığı gözlemlendi. Ölçümler arası korelasyon testinde, talus ölçümünde yaş ile negatif korelasyon değeri tespit edildi. (korelasyon değeri: -0.139-099). Sonuç olarak, klinik açıdan önemli olan bu bölgenin radyolojik ölçümleri, cinsiyet ve ırk ayrımı için alternatif bir yöntem olarak kullanılabilir.

Anahtar sözcükler: *Calcaneus, Diskriminant analizi, Tek tırnaklı, Uzuvlar, Radyometrik, Talus*

How to cite this article?

Olgun Erdikmen D, Gundemir O, Pazvant G, Dayan MO, Duro S, Hartoka H, Pérez W, Parkan Yaramis C: Determination of gender and breed in Arabian horses and thoroughbred horses using radiography of the tarsal region. *Kafkas Univ Vet Fak Derg,* 27 (6): 795-802, 2021.
DOI: 10.9775/kvfd.2021.26337

(*) Corresponding Author

Tel: +90 212 473 7070/17101 Cellular Phone: +90 530 645 7484
E-mail: cparkan@iuc.edu.tr (Ç. Parkan Yaramış)

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Introduction

Using direct bone and living material measurements in taxonomy studies, the differences between sex and species have been revealed for years. However, in recent years, a different perspective has been brought to these studies by using the features of imaging systems. One of its most important features is that it can be sampled on live animals. Later, this data can be followed up on live animals. It is also ethically more advantageous compared to dissection studies. Moreover, thanks to modeling studies, tissue and bone sampling of endemic species can be made and these data can be stored electronically. In addition, with these studies in basic disciplines, results directly related to clinical sciences can be obtained. Because of these features, studies conducted with imaging systems in animals are preferred over the classical dissection method [1-4].

Tarsal articulation is a pelvic limb joint which is composed by cochlea of the tibia, fibula, tarsal and metatarsal bones. The number of tarsal bones is different depending on the animal species, which is six in the horses. The proximal tarsal row is composed by talus and calcaneus, the last is located in plantar and lateral side of the talus. This joint is clinically important in horses, especially Osteochondritis dissecans is an important pathological condition that causes lameness in horses. The radiological image of the tarsal region is very important in the diagnosis of most such diseases and pelvic limb lameness [5,6].

Radiological images have been used in studies such as determining the normal posture position of animals as well as determining bone-joint relations. Gonçalves et al.[7] obtained the parameters related to hoof balance using healthy animals in his study and provided the radiologically based database on this subject. Dorner et al.[8] on the other hand, conducted a radiological study in horses to explain the relationship between the distal phalanx angle and the radiological condition of the navicular bone.

There are studies examining the differences between genders in studies on the tarsal joint. Studies have been conducted on humans and measurements of gender differences have been reported [1,3,4]. The hypothesis of this study is that there are differences between two different horse breeds as well as between the sexes as examined the tarsal joint radiographs.

Material and Methods

Ethical Statement

The study was approved by the Local Ethics Committee of Faculty of Veterinary Medicine, İstanbul University-Cerrahpaşa (Approval no: 2020/41).

Animals

In this research, 213 horses from 2 to 12 years old and without pathological conditions in their tarsal joints were used. Radiographic images of the tarsal joint of the left pelvic limb of Arabian horses (30 females, 57 males) and Thoroughbred horses (57 females, 69 males) were taken from the lateromedial direction. Images were taken while the cassette was in contact with the tarsal joint as the X-ray beam was 70 cm distance. X-ray images were taken at the Istanbul horse Hospital of the Jockey Club of Turkey. Gierth X-ray (model TR90/30) model device was used.

Radiometric Analysis

In the study, a single image taken from the lateral direction was used for each animal. Nine measurements were made over the X-ray image of the tarsal joint *(Fig. 1)*. In addition, two angle values were recorded in the same image *(Fig. 2)*. Measurements were taken on the computer using the Radiant DICOM Viewer (version 2020.2.2) [4,9].

Cranial calcaneus length (CCL): The distance between the highest point of the tuber calcanei and the coracoid process of calcaneus.

Maximum calcaneus length (MCL): The distance between the highest point of the tuber calcanei and the most dorso-distal point of the calcaneus base.

Calcaneal body depth (CBD): The shortest distance between cranial and caudal border of the calcaneus body.

Tuber calcaneus length (TCL): The maximum length of the tuber calcaneus.

Cochlea of tibia´s depth (CTD): The distance from the most cranial margin of the distal part of the tibia to the most caudal border of the cochlea tibia.

Facies articulares talares´s length (FTL): The length between the distal endpoints of the CCL (Cranial calcaneus length) and MCL (Maximum calcaneus length).

Metatarsus depth (MD): The distance from the most cranial margin of the third metatarsal bone to the caudal margin of the fourth metatarsal bone.

Talus ridge´s length (TRL): The distance from the proximal to distal end point of talus ridge.

Talus high (TH): The distance from the talus tuberosity to the deepest point of the cochlea tibia´s.

Proximal angle (PA): The angle between the line drawn from most dorsal point of tuber calcaneus to the coracoid process and the line drawn in the direction of facies articulares talares´s length (FTL).

Distal angle (DA): The angle between the line drawn from most dorsal point of tuber calcaneus to the most dorso-distal point of the calcaneus base and the line drawn in the direction of facies articulares talares´s length (FTL).

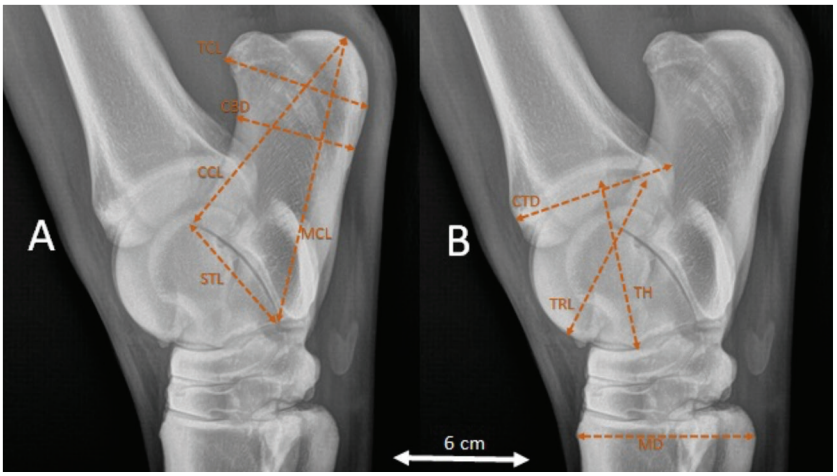

Fig 1. Measurement points (The x-ray image is of a 3-year-old female Thoroughbred horse) **Cranial calcaneus length (CCL):** The distance between the highest point of the tuber calcanei and the coracoideus process of calcaneus**, Maximum calcaneus length (MCL):** The distance between the highest point of the tuber calcanei and the most dorso-distal point of the calcaneus base, **Calcaneal body depth (CBD):** The shortest distance between cranial and caudal border of the calcaneus body, **Tuber calcaneus length (TCL):** The maximum length of the tuber calcaneus, **Cochlea of tibia´s depth (CTD):** The distance from the most cranial margin of the distal part of the tibia to the most caudal border of the cochlea tibia, **Facies articulares talares´s length (FTL):** The length between the distal endpoints of the CCL (Cranial calcaneus length) and MCL (Maximum calcaneus length), **Metatarsus depth (MD):** The distance from the most cranial margin of the third metatarsal bone to the caudal margin of the fourth metatarsal bone, **Talus ridges length (TRL):** The distance from the proximal to distal end point of talus ridge, **Talus high (TH):** The distance from the talus tuberosity to the deepest point of the cochlea tibia´s

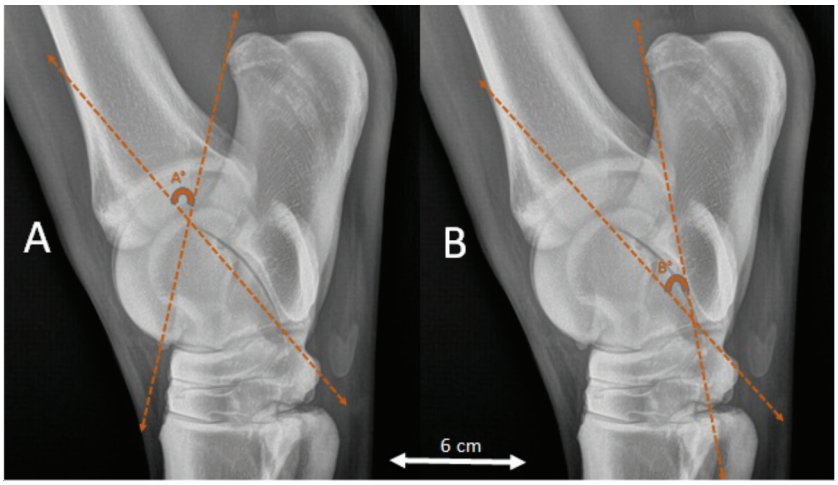

Fig 2. Angle measurements (The x-ray image is of a 3-year-old female British horse) **A: Proximal angle (PA):** The angle between the line drawn from most dorsal point of tuber calcaneus to the coracoid process and the line drawn in the direction of facies articulares talares´s length (FTL), **B: Distal angle (DA):** The angle between the line drawn from most dorsal point of tuber calcaneus to the most dorso-distal point of the calcaneus base and the line drawn in the direction of facies articulares talares´s length (FTL)

Statistical Analysis

Three different groups were created for statistical analysis. In the first group, male and female distinction for Arabian horse was examined. In the second group, male and female distinction was examined for the Thoroughbred horse. In the third group, the differences between the Arabian horse and the Thoroughbred horse were examined, ignoring the gender difference. T test was used to reveal the difference between the two breeds. Discriminant analysis was applied for each group among themselves. Functions in the discriminant analysis were written as formulas. Eigenvalue and Wilks Lambda values were taken. Correctly classified rates received. In addition, the correlations between the data were also taken and those that were statistically significant were indicated in the table. SPSS (version 22) was used for discriminant analysis, correlation test and T test. Visualizing the distribution of samples as a result of

discriminant analysis and for correctly classified values, the statistics program was obtained using Past (4.01).

Results

The mean values, standard deviations and the statistical differences for both Arabian and Thoroughbred horses are presented in *Table 1*. It was seen that only the TRL value made a statistical difference in gender discrimination for Arabian horses (P<0.05). In Thoroughbred horses, it was seen that only the MCL value was a determinant in gender discrimination (P<0.05). The results of the angle values received from this study were not an important factor in gender determination. The study showed that despite the sex differences between the two horse breeds it was observed that all measurements performed, except MCL length play a key role in determining the breed.

Distal angle (DA) measurement was also found to be a determining element in differentiating between these two breeds.

Discriminant analysis was performed for sex determination for both Arabian and Thoroughbred horses. The distribution of Arabian and Thoroughbred horses as a result of the discriminant analysis is plotted in *Fig. 3*.

The Wilks Lambda value between the sex of Arabian horses was 0.685 and the eigenvalue was 0.459.

Discriminant function score equation for stepwise analysis in sex determination of Arabian horse (Group centroids: F: 0,923, M: -0,486) is:

D: (0.413) x CCL + (1.086) x MCL + (-0.904) x CBD + (-0.122) x TCL + (1.996) x CTD + (-1.242) x FTL + (-.651) x MD + (-3.496) x TRL + (1.108) x TH + (0.026) x PA+ (0.107) x DA -1.887.

The Wilks Lambda value between the sex of Thoroughbred horses was 0.851. The eigenvalue was 0.174.

Discriminant function score equation for stepwise analysis in gender determination of Thoroughbred horses (Group centroids: F: 0,456, M: -0,377) is:

Table 1. *Female and male horses measurement values, averages and standard deviations (t test)*

Measurement (cm)	Arabian Horses			Significant	Thoroughbred Horses		
	Female N:30	Significant	Male N:57		Female N:57	Significant	Male N:69
CCL	9.97±0.45	NS	9.83±0.43	NS	10.26±0.53	NS	10.16±0.48
MCL	12.16±0.58	NS	12.02±0.58	***	12.76±0.57	*	12.52±0.61
CBD	5.34±0.23	NS	5.36±0.29	**	5.48±0.36	NS	5.48±0.37
TCL	6.53±0.29	NS	6.54±0.31	***	6.84±0.40	NS	6.80±0.38
CTD	6.53±0.43	NS	6.43±0.43	***	7.04±0.48	NS	7.02±0.46
FTL	5.61±0.39	NS	5.70±0.47	***	6.19±0.42	NS	6.08±0.52
MD	7.20±0.44	NS	7.17±0.50	***	7.87±0.45	NS	7.77±0.44
TRL	6.54±0.43	*	6.77±0.36	***	7.43±0.54	NS	7.54±0.54
TH	7.08±0.36	NS	7.12±0.35	***	7.83±0.44	NS	7.76±0.45
PA	53.85±4.84	NS	52.97±5.20	NS	52.85±4.90	NS	52.80±4.42
DA	31.20±3.19	NS	30.33±3.36	**	29.34±2.92	NS	29.02±2.80

NS: *Non significant, * P<0.05, ** P<0.01, *** P<0.001*
CCL, *cranial calcaneus length;* ***MCL,*** *maximum calcaneus length;* ***CBD,*** *calcaneal body depth;* ***TCL,*** *tuber calcaneus length;* ***CTD,*** *cochlea of tibia´s depth;* ***FTL,*** *facies articulares talares´s length;* ***MD,*** *metatarsus depth;* ***TRL,*** *talus ridge´s length;* ***TH,*** *talus high;* ***PA,*** *proximal angle;* ***DA,*** *distal angle*

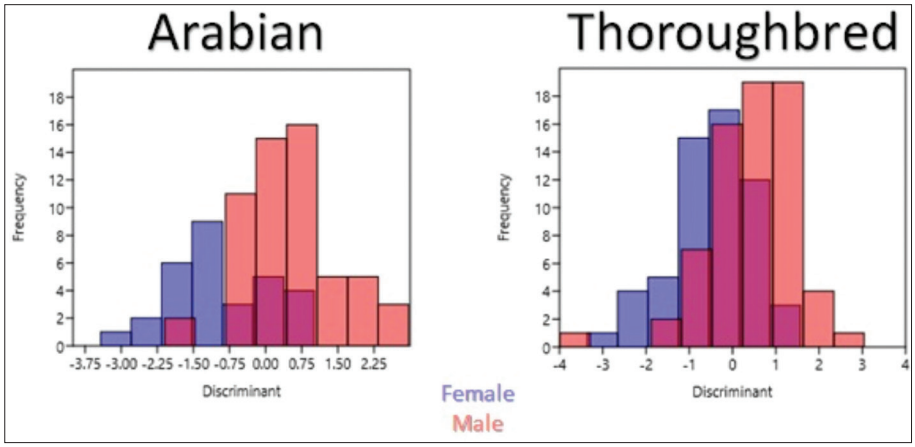

Fig 3. Distribution of female male discriminant analysis result in Arabian horses and Thoroughbred horses

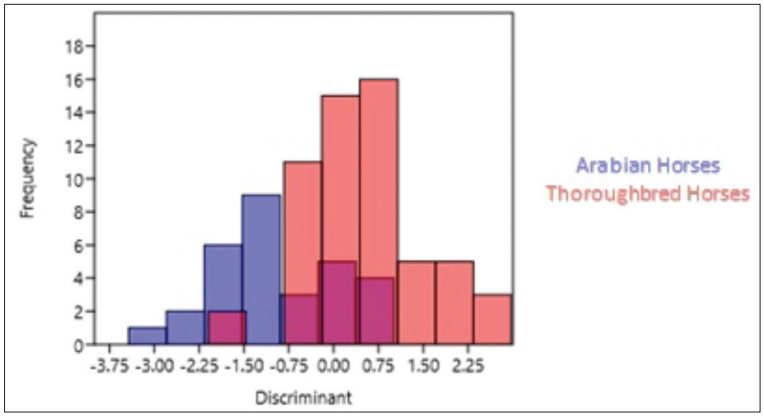

Fig 4. Distribution of discriminant analysis results between Arabian horses and Thoroughbred horses without discriminating between females and males

Table 2. *Confusion matrix. Percentage of initial classifications that were correct, shown by sex*

Breed	Sex	Female	Male	Total	Correctly Classified
Arabian	Female	21	9	30	71.26%
	Male	16	41	57	
Thoroughbred	Female	37	20	57	66.67%
	Male	22	47	79	

Percentages within rows sum to 100%

Table 3. *Confusion matrix. Percentage of initial classifications that were correct, shown by breed*

Breed	Arabian	Thoroughbred	Total	Correctly Classified
Arabian	81	6	87	88.26%
Thoroughbred	19	107	126	

Percentages within rows sum to 100%

D: (-1.499) x CCL + (1.973) x MCL + (-1.263) x CBD + (-1.88) x TCL + (0.685) x CTD + (0.561) x FTL + (0.866) x MD + (-1.544) x TRL + (0.899) x TH + (-0.036) x PA + (0.394) x DA - 21.491.

The distribution of individuals is given in *Fig. 4,* as a result of the discriminant analysis performed between the Arabian and the Thoroughbred horses regardless of gender. The Wilks Lambda was 0.395 and the eigenvalue was 1.530. Discriminant analysis results showed that the most determinant measurements were in the talus values (TRL and TH).

Discriminant function score equation for stepwise analysis between the two breeds. (Group centroids: Arabian horse: -1.482, Thoroughbred horses: 1.023) is:

D: (-0.582) x CCL + (-0.112) x MCL + (-1.854) x CBD + (-0.252) x TCL + (0.431) x CTD + (1.179) x FTL + (1.561) x MD + (1.208) x TRL + (0.402) x TH + (0.023) x PA + (-0.36) x DA – 14.543.

Using these measurements, it was observed that only 71.26% of Arabian horses were accurately classified between males and females. Whereas in Thoroughbred horses only 66.67% of them were correctly classified as males and female *(Table 2)*. The results showed that the exact differentiation of Arabian and Thoroughbred horse breeds was 88.26% regardless of the gender differences *(Table 3)*.

Correlation values between measurements results are given in *Table 4*. It was observed that age had a negative correlation with MCL, CTD, MD, TRL, TH and DA values. Among these values, the highest negative correlation value with age was seen in the TRL measurement (correlation value: -0.139). The correlation with the CBD value was positive and its value was higher than the other measurements (correlation value: 0.190). The correlation value between the two angles was positive and statistically significant. The highest correlation between length measurements was between TRL and CTD measurements (correlation value: 0.780). The correlation between the measurement values of MCL, TCL, MD and TH and the values of the angles were all negative.

Discussion

In this study, tarsal radiography of 213 horses was used and the measurement values of the tarsal region were revealed both between sex and breeds. Different measurement characteristics were seen in two breed types. Thoroughbred horses data was found to be higher

Table 4. *Correlation values between measurement values*

Parameters	CCL	MCL	CBD	TCL	CTD	FTL	MD	TRL	TH	PA	DA
Age	.160*	-.001	.190**	.036	-.014	.040	-.079	-.139*	-.099	.021	-.020
CCL		.291**	.291**	.246**	.262**	.184**	.123	.164*	.209**	.092	.070
MCL			.595**	.729**	.513**	.634**	.695**	.430**	.687**	-.284**	-.393**
CBD				.755**	.564**	.550**	.453**	.433**	.554**	.047	-.091
TCL					.575**	.564**	.636**	.507**	.685**	-.225**	-.306**
CTD						.614**	.412**	.780**	.665**	.267**	.066
FTL							.291**	.422**	.577**	.022	-.296**
MD								.513**	.736**	-.288**	-.365**
TRL									.752**	.178**	.037
TH										-.090	-.255**
PA											.798**

** Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level*
CCL, *cranial calcaneus length;* ***MCL,*** *maximum calcaneus length;* ***CBD,*** *calcaneal body depth;* ***TCL,*** *tuber calcaneus length;* ***CTD,*** *cochlea of tibia´s depth;* ***FTL,*** *facies articulares talares´s length;* ***MD,*** *metatarsus depth;* ***TRL,*** *talus ridge´s length;* ***TH,*** *talus high;* ***PA,*** *proximal angle*

than that of Arabian horses. Most of the differences between them were statistically significant. However, it was observed that the tarsal area measurements were not effective in sex discrimination between species. Correlation between joint measurement points was mostly positive. However, the negative correlation between age and tarsal measurement values was striking. This result may give an idea for future studies.

In this study, sex determination was examined within Thoroughbred horses and Arabian horses. While TRL measurement in Arabian horses was discriminatory for gender, it was found that only MCL measurement was determinant for Thoroughbred horses ($P<0.05$). The difference between gender for all other measurement values was statistically insignificant. In studies conducted in humans, it was stated that calcaneus length measurements in male were higher than that of females, and this difference was statistically significant [1,3]. In some measurements, females were found to be higher than males in both Thoroughbred horses and Arabian horses. Also, although the measurement of calcaneal body depth in horses does not play a decisive role for sex differentiation, however this measurement has been reported to play a determining role in terms of gender history in humans [2,4,9]. In addition, the angle values obtained from measurements in the calcaneus revealed that they were not crucial for sex determining in horses, as were the data presented in the literature [10].

The tarsal region is especially important for racing horses in terms of movement biomechanics. Tendo calcaneus communis is directly connected to the tuber calcanei of the calcaneus and plays a role in the active movement of this region. In this study, it was seen that the measurements of the tarsal region in Thoroughbred horses were higher in all of the longitudinal measurements and this difference was statistically significant. It can be said that the tarsal data should be evaluated together with the gait analysis data in order to comment on whether this difference affects active movement between two horse breeds. In another study, as a result of the gait analysis performed between these two horses, it was stated that there were also differences in the walking kinematics of the two horses, and that there were time differences between extensor and flexor muscle movements [11].

In this study, the difference between right and left was not examined, considering the possibility of a difference, only the lateromedial radiological image of the left side was used and it was examined whether there is only a sex and breed difference. There are studies that take symmetrical measurements of the right and left samples using radiographic images and examine the difference between them. In a study, distal phalanx radiological images of 10 Iranian Arabian horses were used and the difference between right and left measurements was examined. In this study, it was reported that there was no statistical difference between right and left measurements [12]. However, Sakaue [2] reported that he found a statistically significant difference between right and left measurements in his study using calcaneus measurements in humans.

There was a positive correlation between the measurements of the tarsal area in Thoroughbred horses and Arabian horses. However, the correlation between these measurements and age was negative. Especially the negative correlation between TRL measure and age was statistically significant. It was observed that TH value, which is another measurement of talus, had a negative correlation related with age. The talus bone is in the middle of the tarsal joint with respect to the calcaneus, and it transmits the animal's pelvic limb load directly to the metatarsal bone. This result can be discussed with exercise

and age. In the literature, the effects of age and exercise on the bones of horses were also examined in studies on this subject. In a study on bone in horses, changes in the microstructure of bones depending on age were mentioned [13]. The authors argued that this change was not affected by sex, but related to age. In another study conducted with British horses, it was stated that exercise and age can cause changes in the bone structure´s [6]. Cruz et al.[14] reported in his study on horses that differences in bone changes on the distal phalanx were as a result of exercise. In a study conducted on Labrador Retrievers dogs, it was found that age had an effect on the talus bone by increasing its density [15]. To make this information more comprehensive, repeated measurements can be made at different ages on the same horses, perhaps in other similar studies which may help to further clarify this idea.

In this study, radiometric measurements of the field region with lateromedial direction shooting were used. In this sense, just like the reference studies used in the article, only the lateral aspect of the calcaneus and talus, which are the main elements of the study, could be evaluated. In future studies, caudal images of calcaneus, which seems to be determinant among species, can be taken and measurements can be made about the width of the tuber calcanei. In addition, measurements of calcaneus can be taken and differences between two species can be revealed osteometrically. However, thanks to the radiographic method, which is the main material of this study, samples were taken from live animals and a large number of sample groups were reached. In this sense, we can say that radiometric measurements are more advantageous than osteometric measurements. In addition, with radiometric measurements, repeated measurements can be taken from the same horses after a certain period of time and repeated analyzes can be performed. In this way, bone development can also be recorded gradually.

In this study, it is seen that the radiological images of the tarsal region play an important role in sex and breed discrimination. It was observed that especially the calcaneus length measurements revealed a statistically significant difference, whereas the angle measurements did not make a significant difference in sex determination. As the results of this study show, radiological measurements of this clinically important region can be used as an alternative method to be used for sex and breed discrimination.

Availability of Data and Materials

The datasets during and/or analyzed during the current study available from the corresponding author on reasonable request.

Acknowledgements

We thank to Jokey Club of Turkey supports the work.

Funding Support

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for profit sectors.

Conflict of Interest

Authors declares that there is no conflict of interests.

Author Contributions

Design of the study: D. Olgun Erdikmen, O. Gundemir and S. Duro. Preparation of the study and data collection: D. Olgun Erdikmen, H. Hartoka, M. O. Dayan. Article writing, data analysis and editing: D. Olgun Erdikmen, W. Pérez, O. Gundemir, C. Parkan Yaramis, G. Pazvant.

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