Effect of inbreeding on the “Club Foot” disorder in Arabian Pureblood horses reared in Italy

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Abstract

Background: “The Club Foot” (or “Mismatched Foot”) is an acquired or congenital flexural deformity of the distal interphalangeal joint, caused by a shortening of the musculotendinous unit of the deep digital flexor tendon.

Aim: The aim of this research was to detect the incidence of the disorder in Arabian Pureblood horses, attempting to understand its causes and to analyze a possible role of inbreeding in its expression. In this breed, in fact, the pathology is widespread because in the environment of origin, the rocky desert, a hard and almost goat's hoof is not disabling so the selection against this disorder has never been done.

Methods: Pedigrees were taken from 141 (reference population = RP) adult Arabian Pureblood horses (51 males and 90 females) belonging to eight Italian different farms during the period 1982–2017. For each horse, the presence or not of the disorder was observed and inbreeding coefficients (F) was performed. Four grades of deformity were considered. Moreover, the environmental condition of each farm was considered: boxes, paddocks, nutrition, orientation, and hoof care and shoeing. The chi-square test was applied. Analysis of variance (ANOVA) was used to test the differences in the average inbreeding coefficient (F) between affected and healthy animals, between sexes and between shod and unshod animals.

Results: Two grades of deformity were observed (I and II) which give less severe manifestations, with 28 females and 25 males (37.59% of the examined horses) showing the disorder. No differences between males and females or between shod (38.29%) and unshod (61.70%) were observed. Environmental conditions do not influence the rate of pathology in the different farms, with a prevalence of the disorder ranging from 7.69% up to 100% on farm. The whole population (WP = RP and its genealogy) included 3,355 records subdivided in seven traced generations. One hundred and eighteen out of 141 horses (RP) were inbred (83.69%). The average inbreeding coefficient (F) in the RP was 0.095. Inbreeding coefficient in RP was <0.05 in only 15 horses (12.71% of inbred), whereas it was >0.25 in 28 horses (23.73% of inbred).

Conclusion: High inbreeding coefficients were observed in all farms and in particularly in affected animals suggesting that high inbreeding coefficients increases the probability that the disorder occurs. Future works may include the study of the hereditability of the character, and the attempt to identify loci associated with the disorder.

Keywords: Arabian Pureblood horses, Club Foot disorder, Inbreeding, Incidence.

Introduction

The Arabians are commonly believed to be one of the oldest and most influential horse breeds in the world (Głażewska, 2004). Arabian Pureblood horse expresses their versatility in competing in several equestrian fields, including racing, western, dressage, reining, endurance, show jumping, eventing, and even driving (Sobczyńska, 2010). Originating in Saudi Arabia, the Arabian horse has been selectively bred by the Bedouin people who need horses with a small and hard hoof to best ride on a sandy terrain with significant amounts of fragmented rocky components (Schiele, 1970). Horses used in equestrian disciplines are subjected to intense exertion, making the athlete’s physical integrity a vital point, in order to optimize performance as well as to limit the onset of traumatic disorders. Several parameters are involved in choosing a subject for sport activities, one of the most important is the absence of any disorder or malformation of the limbs such as the “Club Foot” or “mismatched foot” that is defined as a flexural deformity of the distal interphalangeal joint, caused by a shortening of the musculotendinous unit of the deep digital flexor tendon (Hunt, 2011). According to O’Grady (2012), the hoof capsule is distorted and the palmar angle of the third phalanx is at least 60° or more, and the horse is forced to walk on his toes. Most commonly, this condition affects the forelimbs, usually one, but, sometimes, both. This deformity is believed to result from mal-positioning of the fetus in the uterus, errors in the nutritional management of the mare during gestation, exposure to influence virus or, in some circumstances, a genetic link. In fact, it can be acquired or congenital (O’Grady, 2012). This pathology is widespread in the Arabian Pureblood horse breed because in the environment of origin, the rocky
Pedigrees and observation on the presence or not of the “Club Foot” disorder were taken from 141 adult Arabian Pureblood horses (51 males and 90 females) belonging to eight Italian different farms during the period 1982–2017. Subsequently, the family tree of each considered subject has been reconstructed, allowing to identify common ancestors and lines. The following parameters have been calculated, using the program ENDOG v4.8 (Gutiérrez and Goyache, 2005): the number of full traced generations, the maximum number of generations traced and the equivalent complete generations. The number of inbred, the inbreeding coefficient of the 141 horses (RP= reference population), the average inbreeding coefficients within the farms, within the two sexes and within healthy and affected animals and within shod and unshod animals were performed using CFC software (Sargolzaei et al., 2006). The distribution of inbreeding level in the population were analyzed and 10 different class level of inbreeding were considered: 0 < F ≤ 0.05; 0.05 < F ≤ 0.10; 0.10 < F ≤ 0.15; 0.15 < F ≤ 0.20; 0.20 < F ≤ 0.25; 0.25 < F ≤ 0.30; 0.30 < F ≤ 0.35; 0.35 < F ≤ 0.40; 0.40 < F ≤ 0.45; 0.45 < F ≤ 0.50 (Sargolzaei et al., 2006).

The presence or not of the “Club Foot” disorder was tested following these criteria: alignment of the front hooves, recognition of pathological signs through analysis of hoof axis, presence of dishing on the anterior hoof wall from the coronary band to the toe, hoof width, heel height, frog atrophy, and presence of laminitis symptoms. For each animal the grading system for the stages of the deformity proposed by Redden (2003) was considered (Table 1). Furthermore for each farm, we consider the environmental factors taken into account in the list proposed by O’Grady and Poupard (2003): box (walls, floor, type of bedding, and cleanout procedures); paddock (type of ground and the time spent by the animals within the paddock); nutrition (gross estimate of the type and amount of forages and concentrates); orientation (training activities); hoof care, and shoeing (Table 2).

Chi-square test (SAS-JMP software version 7.0, 2007) was used to verify the differences in the disorder

| Grade | Description |
|-------|-------------|
| Grade I | The palmar angle of the third phalanx is 3 to 5 degrees greater than the opposing foot, with characteristic fullness of the coronary band due to partial luxation (partial dislocation) of the second phalanx bone and coffin bone. |
| Grade II | The palmar angle of the third phalanx is 5 to 8 degrees greater than the opposing foot, with growth rings wider at the heel than at the toe. The heel will not touched the ground once trimmed to normal length. |
| Grade III | The anterior hoof wall is dished-in, with growth rings at the heel twice as wide as on the toe. Radiographically, the third phalanx exhibits demineralization and lipping along the apex. |
| Grade IV | The anterior hoof wall is heavily dished and the palmar angle of the third phalanx is 80 degrees or more; the coronary band has the same height at the heel as at the toe. Radiographically, the third phalanx is rounded due to extensive mineralization and rotation may be present. |
| Farm | Box stall | Paddock | Nutrition | Orientation | Miscellaneous |
|------|-----------|---------|-----------|-------------|----------------|
| 1    | Wooden walls. Walls: concrete. Bedding: straw. Cleanout: once a week. | Medium grain soil, uneven, with rocks and crevices. Mares are kept daily and return to box stalls at evening. Stallions are kept twice a week. | Grass hay. Concentrate: barley, amount based on the animal’s breeding condition. | Breeding. | Poor hoof care on most horses, no shoeing or regular hoof trimming. |
| 2    | Wooden walls. Walls: concrete. Bedding: straw. Cleanout: daily bedding change. | Mixed sand with gravel, even. All horses kept daily, return to box stalls at evening. | Grass hay + alfalfa hay twice a week. Mixed pasture. Concentrate: corn, barley, locust beans, oats, soy, mineral salts, at morning and evening, amount based on breeding condition. | Breeding. |
| 3    | Concrete walls, single boxes for stallions and non-breeders; large box near the pasture for the mares. Floor: concrete. Bedding: dried shavings. Cleanout: daily. | Medium grain soil, uneven, with terracing and gravel in spots. Stallions and horses in training are kept daily, return to box stalls at evening. Mares do not return to stalls. | Grass hay + alfalfa hay twice a week. Mixed pasture. Concentrate: corn, barley, locust beans, oats, soy, mineral salts, at morning and evening, amount based on breeding condition. | Breeding. Trekking. Foal training. Endurance racing. |
| 4    | Concrete and wooden walls. Floor: concrete. Bedding: dried shavings. Cleanout: daily. | Medium grain soil, good leveling, sloping. Mares are kept daily and return to box stalls at evening. Stallions are kept for training 1 hour daily. | Mixed hay. Concentrate: at morning and evening. | Breeding. Endurance racing. Shows. | Good hoof care and shoeing. |
| 5    | Wooden walls. Floor: soil. Shelters near the paddocks. No bedding. | Medium grain soil, even, poor draining. All horses are kept daily. | Mixed hay, mostly alfalfa. Concentrate: foals and mares fodder, also used for stallions in winter; otherwise, 90% oats + 10% barley for stallions. | Breeding. |
| 6    | Walls: concrete and wood. Walls: concrete, stone and tuff. Bedding: shavings. Cleanout: daily. | Medium grain soil, leveled, on hillside. Mares kept daily. Daily hard ground training session for the show horses. | Grass hay. Concentrate: mixed flakes. Three meals a day for foaling mares and horses preparing for shows. Two meals a day for the others. | Breeding. Shows. |
| 7    | Solid concrete; concrete barn walls, wooden partitions, open shelters near the paddocks. Floor: stone, guttered for drainage. | Even clay soil. Horses kept daily, return to box stalls at evening. | Mixed hay with lots of alfalfa. Morning: carrots. About 1–1.5 kg mixed flakes per animal at morning and evening, based on breeding condition. | Breeding. Endurance racing. Trekking. Foal training. | Plastic shoeing on the front hooves. |
| 8    | Concrete and wooden walls. Floor: concrete. | Medium grain soil, even, poor draining. All horses are kept daily. | Mixed hay. Concentrate: at morning and evening. | Breeding |
incidence existing between males and females or between shod and unshod animals and to test if different environmental conditions can influence the rate of pathology in the different farms. Moreover, ANOVA was used to test the differences in the average inbreeding coefficient ($F$) between affected and healthy animals, between sexes and between affected shod and affected unshod animals (SAS-JMP software version 7.0, 2007). The level of significance was $p = 0.05$. Three different models were used with three different variables entered as fixed factors: sex in the first model, affected and healthy animals in the second model, and affected shod and affected unshod animals in the third model.

**Ethical approval**

“Committee on the Ethics of Animal Experiments of Minimally Invasive Surgery Centre” (Italian laws).

**Results**

Out of the total 141 examined subjects (Table 3) forming the basis for our survey, 51 were males and 90 were females; 127 were over 2-year old (45 males and 82 females) and 14 were under 2-year old (six males and eight females). Out the total, 54 were shod animals and 87 were unshod animals.

Fifty-three subjects out of 141 displayed the deformity (37.58% of the whole examined population). Out of those, 25 were males (two were under 2-year old) and 28 were females (two were under 2-year old). The total affected shod animals were 20 (37.00% of shod animals and the total affected unshod were 31 (35.63% of unshod animals). The lesion was observed in both males and females and no significant prevalence by sex or shod/unshod were observed. Two grade of deformity were observed, grade I and II (grade I: 42 animals; grade II: 11 animals) that give less severe manifestations in comparison to the deformity of grade III and grade IV (Table 1). By comparing the pedigree charts of subjects kept in the eight farms, we have been able to observe the presence (or absence) of affected subjects and phenotypically healthy subjects in the family trees, and to know the frequency of recurrence of the character. It also emerges that even phenotypically healthy parents will sire affected progeny. Moreover, a thorough examination of the data shows that the lesion is not displayed in every generation. As an example, the graphical representation of a pedigree of one affected horse of farm 5 is shown in Figure 1.

Table 3. Data from the total sample population: rate of pathology among the sexes, by age and shoeing.

| Animals                                                | Total N° | Males N° | Females N° |
|--------------------------------------------------------|----------|----------|------------|
| Animals examined, or with known hoof conformation      | 141      | 51       | 90         |
| Total animals 2 years old or younger                    | 14       | 6        | 8          |
| Total animals over 2 years old                         | 127      | 45       | 82         |
| Total healthy animals                                   | 88       | 26       | 62         |
| Total affected animals                                  | 53       | 25       | 28         |
| Grade I                                                | 42       | 20       | 22         |
| Grade II                                               | 11       | 5        | 6          |
| Total shod animals                                      | 54       | 20       | 34         |
| Total affected shod animals                             | 20       | 8        | 12         |
| Total unshod animals                                    | 87       | 31       | 56         |
| Total affected unshod animals                           | 31       | 10       | 21         |
animals (ranging from 0.040 in farm 2 to 0.337 in farm 4). Moreover, significant differences in the average inbreeding value were detected between healthy and affected animals (average $F = 0.042$ in healthy animals, versus average $F = 0.182$ in affected animals; $p < 0.01$). Concerning the presence of affected animals inside the all eight farms although the females are in greater numbers than the males, there are no significant differences among the sexes in the disorder prevalence in farm 1, 2, and 3 (Table 5). In the other farms, there is a higher percentage of affected males (farms 4, 5, and 6) or of females (farm 7). In farm 8, all the animals were males and were all affected. There are no significant differences between shod and unshod animals in the disorder prevalence in the total sampling (Table 3) and in each farm (Table 6) and there are no significant differences in the average inbreeding among affected shod animals and affected unshod animals.

**Discussion**

The “Club Foot” is a biological event challenging the history and the daily practice of horse breeding, and giving us significant insight into the entrepreneurial difficulties facing the breeders and the issues of social adaptation deriving from this condition. This proves particularly true and interesting of a world that the methods of modern experimental science have yet failed to penetrate efficiently. The empirical observations, the expectations, the craft, and the skills are all part of a cultural context that has not produced a tentative solution for decades. A genetic component must also be considered for acquired flexure deformities, as some mares consistently produce foals that develop a flexural deformity in the same limb as the dam or grand dam in which a similar deformity is present (O’Grady, 2012). In the present study, we have studied different families in order to lay a foundation for the naturalistic

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**Table 4. Analysis of inbreeding by farm.**

| Farm | N° Total animals | Average $F$ | N° Males | N° Females | N° Inbred animals | Average $F$ in the inbred | $F$ max | % Inbred animals per farm |
|------|------------------|-------------|----------|------------|------------------|--------------------------|---------|--------------------------|
| N° 1 | 32               | 0.100       | 12       | 20         | 31               | 0.100                    | 0.230   | 96.85                    |
| N° 2 | 18               | 0.030       | 5        | 13         | 13               | 0.050                    | 0.120   | 72.22                    |
| N° 3 | 26               | 0.040       | 11       | 15         | 24               | 0.060                    | 0.200   | 92.31                    |
| N° 4 | 29               | 0.080       | 5        | 24         | 20               | 0.110                    | 0.500   | 68.96                    |
| N° 5 | 3                | 0.110       | 2        | 1          | 3                | 0.110                    | 0.210   | 100.00                   |
| N° 6 | 14               | 0.050       | 6        | 8          | 8                | 0.100                    | 0.200   | 57.14                    |
| N° 7 | 13               | 0.170       | 4        | 9          | 13               | 0.170                    | 0.410   | 100.00                   |
| N° 8 | 6                | 0.180       | 6        | 0          | 6                | 0.180                    | 0.370   | 100.00                   |
| **Total** | **141** | **0.095** | **51**   | **90**     | **118**          | **0.110**                | **0.500** | **83.69**               |
A hypothesis that a genetic etiology can be found, which is responsible for the expression of a characteristic within a population. The high percentage of inbred in the RP and in each farm and the presence of many animals with high inbreeding coefficients values (higher than 3.125% and even 40.00%), showed an excessive use of the inbreeding as a mating method. In fact we must consider that values higher than 3.125% resulted from the mating of two animals sharing a single grandparent, while values higher than 40.00% correspond to the closest inbreeding, when breeding of brothers with sisters or parents with descendants takes places in several successive generations. High inbreeding coefficients were observed also in a previous research conduct on the Spanish Arab Horse (Cervantes et al., 2008).

Many other studies have reported inbreeding results in horse breeds and their analyses show that the mean coefficient of inbreeding differs for different breeds and within the same breed in different countries. The average coefficient of inbreeding observed in this population was similar to findings by Sairanen et al. (2009) for Standardbred trotters, and by Avdi and Banos (2008) for the endangered Skyros breed, but it was slightly smaller than those reported by Votrava-Vydrová et al. (2016) in grey horses and in Black horses. Moreover, the average inbreeding values observed in some farms were higher than those found in some dog breeds (Ciampolini et al., 2013; Cecchi et al., 2018) that use commonly and notoriously inbreeding as a mating method. The main result that emerges in this research is certainly the great difference in the average value of inbreeding between healthy and affected animals that lets us to identify an important role of inbreeding for the expression of the disorder: high inbreeding coefficients increases the probability that the disorder occurs. Also, Dolvik and Klemetsdal (1994) found that Arthritis in the carpal joints of Norwegian trotter increased the probability of the disease of 6.70% and 12.30% among horses with, respectively, lower or higher inbreeding coefficients than the average. As well-known inbreeding can increase genetic diseases so we could assume a formal genetic pattern compatible with an autosomal recessive monogenic inheritance. As inbreeding can cause also “Inbreeding Depression,” the polygyny of the condition cannot be ruled out entirely, since that will only be possible when the molecular identity of a single responsible gene can be fully described; it is still true that the our data do not point toward the influence of husbandry conditions nor of shoeing practices.

Actually, it emerges indisputably that the rate of pathology in farms with optimal environmental conditions is not significantly different from the farms where the quality of husbandry is limited by competence or budget reasons. If we analyze the rate of pathology, we find that the percentage of affected subjects has no relation between the disorder and shod or unshod animals. The same applies to the hoof care, the nutrition, the type of soil that the animals tread.

### Table 5. Rate of the pathology and average inbreeding coefficient (F) in healthy and affected animals by sex and farm.

| Farm | N° Total affected Males per farm | % Healthy Males | Average F in healthy Males | N° Total affected Females per farm | % Healthy Females | Average F in healthy Females | N° Total affected Males | % Affected Males per farm | Average F in affected Males | N° Total affected Females | % Affected Females per farm | Average F in affected Females |
|------|---------------------------------|----------------|--------------------------|---------------------------------|-----------------|-----------------------------|------------------------|--------------------------|--------------------------|------------------------|--------------------------|-----------------------------|
| N° 1 | 15                              | 0.110          | 17                       | 53.33                           | 0.020           | 6                           | 0.040                  | 6                        | 0.190                    | 17                      | 53.33                    | 0.020                       |
| N° 2 | 12                              | 0.020          | 6                        | 66.67                           | 0.040           | 6                           | 0.040                  | 6                        | 0.190                    | 17                      | 53.33                    | 0.020                       |
| N° 3 | 2                               | 0.110          | 24                       | 7.69                            | 0.190           | 24                          | 0.377                  | 24                       | 0.377                    | 24                      | 7.69                     | 0.190                       |
| N° 4 | 5                               | 0.215          | 3                        | 17.24                           | 0.037           | 3                           | 0.037                  | 3                        | 0.037                    | 3                      | 17.24                    | 0.037                       |
| N° 5 | 2                               | 0.150          | 9                        | 35.71                           | 0.150           | 9                           | 0.085                  | 9                        | 0.085                    | 9                      | 35.71                    | 0.150                       |
| N° 6 | 6                               | 0.180          | 88                       | 53.84                           | 0.215           | 88                          | 0.042                  | 88                       | 0.042                    | 88                     | 53.84                    | 0.215                       |
| N° 7 | 6                               | 0.180          | 88                       | 53.84                           | 0.215           | 88                          | 0.042                  | 88                       | 0.042                    | 88                     | 53.84                    | 0.215                       |
| N° 8 | 6                               | 0.180          | 88                       | 53.84                           | 0.215           | 88                          | 0.042                  | 88                       | 0.042                    | 88                     | 53.84                    | 0.215                       |
| Total| 53                              | 0.182          | 28                       | 37.59                           | 0.042           | 28                          | 0.042                  | 28                       | 0.042                    | 28                     | 37.59                    | 0.042                       |
daily, the number of hours that the animals spend in the paddocks and in the box stalls, and the degree of inbreeding and the type of sport activities that the animals practice. We can and do wish that the results of the present study will lay the foundation for further research, to be carried out by extending the study to the hereditability of the characteristics, and to realize studies based on either candidate genes or genome-wide association studies with the attempt to identify loci associated with the disorder.

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Conflict of interest
The authors declare that there is no conflict of interest.

Author contributions
L.C.: concept/design, acquisition of data, drafting of the manuscript, assembled tables and figures, and approval of the article. A.P.: concept/design, critical revision of the manuscript, and approval of the article. C.R.: critical revision of the manuscript and approval of the article. C.F.: concept/design, statistical analysis, drafting of the manuscript, assembled tables and figures, critical revision of the manuscript, and approval of the article.

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| Farm | N° Shod animals | N° affected shod animals | % Affected shod animals per farm | Average F in shod animals | N° Unshod animals | N° affected unshod animals | % Affected unshod animals per farm | Average F in unshod animals |
|------|----------------|-------------------------|---------------------------------|--------------------------|----------------|-------------------------|---------------------------------|--------------------------|
| N°1  | 10             | 4                       | 40.00                           | 0.087                    | 22            | 8                       | 36.36                           | 0.106                    |
| N°2  | 6              | 4                       | 66.67                           | 0.062                    | 12            | 8                       | 66.67                           | 0.029                    |
| N°3  | 5              | 0                       | 0.00                            | 0.072                    | 21            | 2                       | 9.50                            | 0.033                    |
| N°4  | 22             | 4                       | 18.18                           | 0.108                    | 7             | 1                       | 14.28                           | 0.057                    |
| N°5  | 1              | 1                       | 100.00                          | 0.060                    | 2             | 2                       | 100.00                          | 0.135                    |
| N°6  | 2              | 1                       | 50.00                           | 0.000                    | 12            | 4                       | 33.33                           | 0.058                    |
| N°7  | 3              | 1                       | 33.33                           | 0.210                    | 10            | 5                       | 50.00                           | 0.045                    |
| N°8  | 5              | 5                       | 100.00                          | 0.142                    | 1             | 1                       | 100.00                          | 0.370                    |
| Total| 54             | 20                      | 37.04                           | 0.100                    | 87            | 31                      | 35.62                           | 0.075                    |
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