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Short Communication

# Prevalence of twin foaling and blood chimaerism in purebred Spanish horses

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ABSTRACT

Twin foaling is associated with chimaerism in several domestic species and is recognised in horses. In this study, 21,097 purebred Spanish (Pura Raza Español) horse births from the 2015 to 2016 breeding season were investigated for chimaerism. Twin foaled and chimaeric individuals were assessed on the basis of foaling records, short-tandem repeat (STR) parentage test results and a sex-linked STR-based technique. Fourteen twin pregnancies with 23 twin foals born alive were identified (0.066% twin foaling prevalence), including five blood chimaeric cases (21.7%; overall prevalence 0.011%), suggesting that this genetic condition is extremely low in horses. Furthermore, no true chimaeras were detected. This is the first large scale study analysing the occurrence of chimaerism in a horse population and the first assessment of twin foaling in purebred Spanish horses.



Chimaerism is a widely described condition in livestock species, produced either by the exchange of haematopoietic stem cells through placental circulation between dizygotic twins (blood chimaerism) or by the fusion of two zygotes or embryos into a single individual at the very early stages of development (true chimaerism) (Padula, 2005). Although true and blood chimaerisms have already been reported in horses (Dunn et al., 1970), to our knowledge there is only one report of blood chimaerism in which both twin foals were born alive (Juras et al., 2010).

Chimaerism has been proposed as an important sex chromosomal syndrome, as well as a common cause of sterility, in several domestic species, but has not been investigated in detail in horses (Padula, 2005). The most comprehensive study assessing chimaerism in horses was performed in fetuses by Bouters and Vandeplassche (1972), who demonstrated that 44% of twin pregnancies carried this condition. Although twin conception rates are 3.5–4.5% in several horse breeds, rates of chimaerism and the prevalence of twin foaling have not been reported in horses. This is probably because twin pregnancies in mares are considered to be pathological and frequently are associated with abortions in the last trimester. The prevalence of chimaerism in large horse populations has not been determined.

The use of short tandem repeat markers (STRs) for determining chimaerism in horses was originally proposed by Kakoi et al. (2005) and more recently validated in purebred Spanish (Pura Raza Español) horses by Anaya et al. (2017). This methodology allows the simultaneous analysis of multiple individuals more rapidly and less expensively than traditional karyotyping. This approach was incorporated by the National Purebred Spanish Horse Breeders' Association[1] in 2015 for the genetic assessment of pure Spanish horses to detect chromosomal abnormalities in young foals before they are incorporated to the studbook. Animals to be included in the studbook are screened on the basis of molecular pedigree testing and individuals with abnormal genetic profiles are evaluated with this new methodology.

In this study, we assessed the prevalence of twin foaling and

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[1] See: www.ancee.es (accessed 15 February 2018).

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**Table 1**
Identification, sex, birth date, type of birth and chimerism status of twin purebred Spanish horses included in this study.

| Foal | Birth date | Type of birth | Chimaerism status |
|---|---|---|---|
| Filly 1 | 8 January 2015 | Normal twins | Normal |
| Colt 1 | 8 January 2015 | Normal twins | Normal |
| Filly 2 | 10 January 2015 | Male twin stillborn | Chimaeric |
| Filly 3 | 5 March 2015 | Normal twins | Normal |
| Colt 3 | 5 March 2015 | Normal twins | Normal |
| Filly 4 | 8 March 2015 | Male twin stillborn | Normal |
| Filly 5 | 15 March 2015 | Normal twins | Normal |
| Colt 5 | 15 March 2015 | Normal twins | Normal |
| Filly 6 | 23 May 2015 | Normal twins | Normal |
| Colt 6 | 23 May 2015 | Normal twins | Normal |
| Filly 7a | 23 May 2015 | Normal twins | Normal |
| Filly 7b | 23 May 2015 | Normal twins | Normal |
| Filly 8a | 30 June 2015 | Normal twins | Chimaeric |
| Filly 8b | 30 June 2015 | Normal twins | Chimaeric |
| Filly 9 | 17 February 2016 | Normal twins | Chimaeric |
| Colt 9 | 17 February 2016 | Normal twins | Chimaeric |
| Filly 10 | 16 March 2016 | Normal twins | Normal |
| Filly 10 | 16 March 2016 | Normal twins | Normal |
| Filly 11a | 29 April 2016 | Normal twins | Normal |
| Filly 11b | 29 April 2016 | Normal twins | Normal |
| Colt 12 | 01 June 2016 | Female twin stillborn | Normal |
| Filly 13a | 21 August 2016 | Normal twins | Normal |
| Filly 13b | 21 August 2016 | Normal twins | Normal |

chimaerism in purebred Spanish horses by screening 21,097 foaling reports belonging to the last two reproductive seasons (10,156 from 2015 and 10,941 from 2016). DNA parentage testing was performed in order to confirm the presumed record-based twin foals using 17 STRs recommended by the International Society of Animal Genetics[2] for paternity testing and individual identification (see Appendix: Supplementary Table 1). DNA was isolated from blood and hair using the Blood and Tissue Genomic DNA Extraction Kits (Canvax), respectively. Fragment amplification was performed by multiplex PCR using the StockMarks for Horses 17-Plex Genotyping Kit (Thermofisher Scientific) and further genotyped by capillary sequencing. Foals with abnormal genetic results, i.e. showing three or more alleles, were diagnosed as presumptive chimaeras. In such cases, additional genotyping was performed on blood and hair samples using seven sex-linked STRs (Anaya et al., 2017) to determine the type of chimaerism (blood or true chimaerism).

Fourteen twin deliveries were detected and confirmed in 2015 and 2016, establishing the twin foaling rate at 14/21,097 (0.066%). Five foals, derived from four different pregnancies, were reported as stillborn or died in the first 48 h; samples from these foals were not available for analysis. Of the three live foals derived from those births, one was a chimaeric filly, one was a normal filly and the other a normal colt. Four additional twin brothers were detected as chimaeric among the remaining 10 twin foals, representing a total prevalence of 21.7% (5/23 analysed foals) among twin deliveries and 0.011% in the entire population (Table 1). The results obtained from blood (chimaeric tri-allelic STR profile) and hair (normal DNA profile according to phenotypic sex) DNA samples demonstrated that all of the studied cases were blood chimaeras.

To our knowledge, the present study is the first comprehensive assessment of the prevalence of twin births in horses from any breed or population. Jeffcott and Whitwell (1973) reported 62 twin foaling events among several breeds of horses, but without determining their prevalence or the occurrence of chimaerism. Higher twin conception rates (up to 5%) have been reported in other breeds of horses (Allen et al., 2007), suggesting that a large percentage of twin pregnancies are unsuccessful in horses.

[2] See: http://www.isag.us/committees.asp (accessed 15 February 2018).

Likewise, there are no previous large-scale studies analysing the occurrence of chimaerism in horses from any breed or population. In the present study, we found that 21.7% of twin foals born alive were chimaeric, which is significantly lower than the 44% previously reported by Bouters and Vandeplassche (1972) in aborted fetuses. Additionally, Bugno et al. (2007) performed the only population study to date evaluating chromosomal abnormalities in horses, reporting a prevalence of chimaerism close to 0.2% in autochthonous Polish breeds. However, our results, which include the analysis of complete delivery records of purebred Spanish horses worldwide during two recent reproductive seasons (2015 and 2016) with a highly sensitive methodology, suggest that the prevalence of chimaerism could be lower than previously reported, or that it could be affected by the breed analysed.

Most of the blood chimaeric horses reported to date were associated with a normal morphology of the reproductive tract and gonads, even when one of the cell lines detected was aberrant (Demyda-Peyrás et al., 2014). Some of the individuals studied here have already produced offspring, indicating that they are fertile (data not shown), but the small number analysed was not sufficient to determine whether chimaerism could affect reproductive capacity in horses, as has been described in other species. There would be value in a study analysing the reproductive capacity of chimaeric horses.

## Conflict of interest

None of the authors of this paper has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the paper. All of the data and samples employed in this study were provided by the Asociación Nacional de Criadores de Caballo de Pura Raza Española, which is authorised by the owners to employ the genetic and morphological data of the horses enrolled in their genealogical book for non-commercial scientific purposes.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.tvjl.2018.02.011.

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