The carrier prevalence of severe combined immunodeficiency, lavender foal syndrome and cerebellar abiotrophy in Arabian horses in South Africa

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Summary

Reasons for performing study: The carrier prevalence of severe combined immunodeficiency (SCID), lavender foal syndrome (LFS) and cerebellar abiotrophy (CA) in Arabian foals in South Africa was determined in order to quantify the potential impact of these conditions locally. Furthermore, the carrier prevalence of SCID prior to and following the introduction of a genetic test was compared to evaluate the effect of testing in the population.
Objectives: To estimate the carrier prevalence of SCID, LFS and CA in registered purebred Arabians born in South Africa in the 2004/5 and 2009/10 foaling seasons and compare the changes in prevalence in these disorders between the 2 groups of foals.
Study design: Cross-sectional survey.
Methods: Samples were collected from individuals randomly selected from 2 populations of purebred Arabian foals born during the 2004/5 and 2009/10 foaling seasons. Genetic testing for SCID, LFS and CA was performed on DNA extracts using specific polymerase chain reactions, with the products being analysed using fragment analysis on a genetic analyser.
Results: The carrier prevalence of LFS and CA for the 2009/10 season was 11.7% (95% confidence interval [CI] 7.6–17.0%) and 5.1% (95% CI 2.5–9.1%), respectively, with no statistically significant change in prevalence between the 2004/5 and 2009/10 foaling seasons. However, the carrier prevalence of SCID was found to have decreased significantly from 6.4% (95% CI 4.8–8.3%) in the 2004/5 foals to 3.4% (95% CI 2.2–5.1%) in the 2009/10 foals (P = 0.009).
Conclusions: The results of this study indicate that genetic screening of Arabian horses for SCID may have played a role in significantly reducing the carrier prevalence within the breeding population and thereby reducing the birth of clinically affected individuals. This study provides an indication of the positive effect of genetic screening for specific conditions in horses.

Keywords: horse; Arabian; prevalence; severe combined immunodeficiency; lavender foal syndrome; cerebellar abiotrophy

Introduction

Severe combined immunodeficiency (SCID) of Arabian horses is a primary immunodeficiency disorder characterised by a lack of functional B and T lymphocytes, which results in a profound susceptibility to infectious agents and death before the age of 6 months. The genetic basis of the condition is a 5 base pair deletion in the gene coding for DNA-dependent protein kinase (DNA-PK) catalytic subunit (cs) [1]. The carrier prevalence of SCID in Arabian horses varies between 8.4% in the USA [2], 1–5% in the UK [3], 7% in Morocco [4], 1.5% in Brazil [4] and 0% in Poland, Slovenia and Iran [4–6].

Lavender foal syndrome (LFS) is a lethal disorder of Arabian foals characterised by a dilute coat colour and a range of neurological signs, including recumbency, opisthotonous, paddling movements and extensor rigidity [7,8]. The genetic basis of LFS is a single base pair deletion in the *MYO5A* gene, which codes for the protein myosin-Va [9,10]. The carrier frequency of LFS in the USA is estimated to be 10.3% in Egyptian Arabian and 1.8% in non-Egyptian Arabian populations [9].

Cerebellar abiotrophy (CA) is a progressive neurological disorder characterised by the degeneration of cerebellar Purkinje cells [11]. Clinical signs of CA usually develop between the ages of 6 weeks and 4 months and include ataxia, hypermetria and intention head tremors [12]. The genetic mutation responsible for the disorder is a single nucleotide polymorphism (G→A) located adjacent to a potential binding site for GATA-2. GATA-2 is a transcription factor involved in expression of MUTYH, a post replication DNA glycosylase [12]. Brault and Penedo [13] estimated the carrier frequency of CA in Arabians in the USA to be 19.7%.

The development of genetic tests for these autosomal recessive conditions has provided a means of identifying carriers of these disorders. This enables breeders to manage breeding programmes and thereby prevent the birth of affected offspring and avoid associated financial losses. Determination of the prevalence of carriers of such disorders in a population allows quantification of the potential impact of the disease on a population. The carrier prevalence of SCID, LFS and CA has not previously been determined in Arabian horses in South Africa. This study reports the carrier prevalence of these 3 disorders among Arabian horses in South Africa as well as the change in prevalence of each disorder between foals born in the 2004/5 and 2009/10 foaling seasons.

Materials and methods

The 2 target populations for this study were registered Arabian foals born during the physiological breeding season in South Africa between 1 August 2004 and 31 July 2005 (2004/5) and between 1 August 2009 and 31 July 2010 (2009/10). The study populations (Table 1) consisted of individuals that had undergone routine DNA profiling and parentage verification as required for registration with the Arabian Horse Society of South Africa through the Veterinary Genetics Laboratory at the University of Pretoria. For each of the 2 seasons, the list of available samples was randomly sorted and, starting from the top of the list, samples were tested for each of the 3 disorders until results had been obtained from approximately 200 foals. In addition, all available SCID genotypes for members of the study populations, derived from routine SCID testing at the Veterinary Genetics Laboratory, were added to the dataset (Table 1). Routine SCID testing was introduced in South Africa in July 2005, while genetic tests for LFS and CA only became available in 2009 and 2011, respectively, and therefore, the temporal effects of genetic testing could only be evaluated for SCID.

Tissue and blood samples were processed using a phenol-chloroform DNA extraction protocol. Sodium hydroxide extraction was used to isolate DNA from hair roots, and whole blood stored on FTA cards[a] underwent DNA extraction as described previously [14]. To determine the SCID, LFS and CA genotypes, polymerase chain reaction (PCR) was performed as 2 separate assays using primers obtained from Life Technologies[b]. Wild-type

TABLE 1: Prevalence of carriers of SCID, LFS and CA among registered Arabian foals born in South Africa during the 2004 and 2009 breeding seasons

Breeding season		2004	2009
Total population size		826	898
SCID	Sample size	800	699
	Proportion of heterozygotes (%)	6.4[a]	3.4[b]
	95% CI* (%)	4.8–8.3	2.2–5.1
LFS	Sample size	203	197
	Proportion of heterozygotes (%)	13.3[c]	11.7[c]
	95% CI* (%)	9.0–18.8	7.6–17.0
CA	Sample size	203	197
	Proportion of heterozygotes (%)	4.9[d]	5.1[d]
	95% CI* (%)	2.4–8.9	2.5–9.1

CA = cerebellar abiotrophy; LFS = lavender foal syndrome; SCID = severe combined immunodeficiency. *Exact binomial 95% confidence interval (CI). [a,b]SCID: P = 0.009 (Fisher's exact test). [c]LFS: P = 0.65 (Fisher's exact test). [d]CA: P = 1.00 (Fisher's exact test).

and mutant CA alleles were amplified using allele-specific PCR [13]. Wild-type and mutant SCID [3] and LFS alleles [10] were amplified as a multiplex PCR using primers described previously. An initial activation step of 95°C for 5 min was followed by 35 cycles of 95°C for 45 s, 56°C for 15 s and 72°C for 15 s, with a final elongation step of 72°C for 10 min. Both PCRs were carried out on a 9800 Fast Thermal Cycler[b], in a total volume of 11 µl per sample consisting of 0.4 µl primer mix, 3.6 µl molecular grade water, 5 µl KAPA2G Robust HotStart ReadyMix[c] and 2 µl of DNA extract at a concentration of approximately 100 ng/µl. Positive and negative controls were included in each run.

A total of 0.5 µl of each PCR product, 10 µl of Hi-Di Formamide[b] and 0.25 µl of GeneScan – 500 LIZ Size Standard[b] were combined prior to capillary electrophoresis on an ABI 3130 *XL* Genetic Analyzer[b]. Fragment sizes of each marker were recorded and analysed using the software programme STRand version 2.4.49[d] [15].

For each disease, carrier prevalence was calculated with exact binomial 95% confidence intervals, and the carrier prevalence among Arabian foals born in 2004/5 was compared with that of foals born 2009/10 using a two-tailed Fisher's exact test. Statistical analysis was done using Stata 12[e], and P<0.05 was considered statistically significant.

Results

For the 2004/5 foals, a total of 349 stallions were used, with a median of 2 foals sired per stallion (range, 1–21; interquartile range, 1–3). During the 2009/10 foaling season, 378 stallions were used, again with a median of 2 foals per stallion (range, 1–34; interquartile range, 1–3). The carrier prevalence of LFS and CA among foals born during the 2009/10 foaling season was 11.7% and 5.1% respectively, with no statistically significant change in the prevalence of these disorders between the 2004/5 and 2009/10 foals. However, the prevalence of SCID was found to have decreased significantly, from 6.4% in the 2004/5 foals to 3.4% in the 2009/10 foals (P = 0.009; Table 1).

Discussion

Lavender foal syndrome of Arabian horses is most commonly observed in Egyptian Arabians [7]. The carrier frequency of LFS in South Africa was higher than estimates by Brooks *et al*. [9], possibly due to a strong Egyptian Arabian influence on breeding programmes in South Africa. Assuming random mating and an infinite population size, approximately 4 LFS-affected foals would be expected per 1000 conceptions. However, within certain breeding lines the risk of producing an LFS-affected foal could be significantly higher. The prevalence of CA, at 5.1%, is lower than the estimate by Brault and Penedo [13], suggesting that fewer carriers of CA have been used as popular sires in South Africa. The marked decrease in the prevalence of SCID since the introduction of a genetic test for this disorder in 2005 is possibly a positive sequel to genetic testing. Complete exclusion of carriers from breeding programmes hastens removal of a disease allele from the population [16]; however, this practice may result in shrinkage of the available gene pool and the possible loss of desirable genes held by carrier individuals. This study did not address specific lines or the influence of founder animals or popular stallions, which may warrant further study.

Authors' declaration of interests

Two of the authors (A.J. Guthrie and C.K. Harper) acknowledge that they are co-inventors of the test for lavender foal syndrome and are employed by the University of Pretoria, which owns the patent on the test that appears in the manuscript.

Ethical animal research

This study was approved by the University of Pretoria's Animal Use and Care Committee according to the South African National Standard (SANS 10386: 2008) for the care and use of animals for scientific purposes.

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Authorship

All authors contributed to the study design, C.J. Tarr, A.J. Guthrie and C.K. Harper were responsible for study execution, and all authors contributed to data analysis and interpretation, preparation of the manuscript and final approval of the manuscript.

Manufacturers' addresses

[a]Whatman Ltd, Maidstone, Kent, UK.
[b]Life Technologies, Johannesburg, Gauteng, South Africa.
[c]Kapa Biosystems, Cape Town, Western Cape, South Africa.
[d]University of California, Davis, California, USA.
[e]StataCorp, College Station, Texas, USA.

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