GENETIC ANALYSIS OF NIGERIAN HORSES USING MICROSATELLITES

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ABSTRACT

To our knowledge this study was the first to analyze the genetic variability of Nigerian horses through genetic markers. Genetic variation in 15 microsatellite loci was examined in 31 Nigerian horses and high levels of genetic variation were observed. The values were similar to those found in Barb horses from Morocco and Algeria.

Keywords: genetic variation, microsatellites, Nigerian horse, *Equus caballus*.

INTRODUCTION

Domestic animal diversity, represented by the wide range of breeds existing nowadays, is essential to sustain and enhance the productivity of agriculture. The genetic diversity found in domestic animal breeds allows farmers to select stock or develop new characteristics or even new breeds in response to changes in the environment, threats of disease, market conditions and social needs, all of which are largely unpredictable. Therefore, the concern with the conservation of genetic variation of livestock breeds has been present in the last decades and genetic markers are being used to assess levels of genetic variation within breeds (e.g. Cothran *et al.*, 2011, Kanshour *et al.*, 2013, Pablo-Gómez *et al.*, 2016).

According to the Domestic Animal Diversity Information System hosted by FAO (http://dad.fao.org/), there are in Nigeria four horse breeds locally adapted: Hausa, Sulebawa, Bornu and Bhirum pony. However, there are no studbooks and breeding is not controlled, so horses are generally referred to as Nigerian local horses to remove any ambiguity.

The Nigerian horses are very comfortable to ride and are mostly used for riding, racing and for Durbar, a biannual ceremony to mark the culmination of the Muslim festivals in the northern part of the country where there is a procession of elaborately dressed horses and horsemen. They also are used in sporting activities (polo tournament), as police mounts, and in the rural areas they are used as work horses on farms. They are not used for milk production but some people in the southern part of the country consume their meat. Traditionally riding a stallion is more prestigious and this has affected the rate at which horse owners breed their horses, making stallions more common among locals. Among racehorse breeders, breeding is mostly done to replace old or injured champion racehorses. Since there are no general breeding strategies and no tools are being used to ensure their orderly and safe use it is essential to characterize these horses genetically in order to be able to contribute to their conservation.

The genetic characterization of a breed is the first step for its conservation and may have implications in future breeding strategies. Microsatellites have proved to be useful to analyse the genetic diversity in several breeds (e.g. Berber *et al.*, 2014, Seo *et al.*, 2016). Therefore, in the present study microsatellites markers were used to characterize the Nigerian horses.

MATERIAL AND METHODS

Hair samples were collected from a total of 31 non registered horses in Sokoto north-western region of Nigeria. Total DNA was extracted from the hair follicles using a Puregene® DNA purification kit according to the manufacturer's instructions.

The DNA typing panel consisted of 15 microsatellites marker loci specific to *Equus caballus* and recommended by the International Society for Animal Genetics: AHT4 and AHT5

(Binns *et al.*, 1995), ASB2 and ASB17 (Breen *et al.*, 1997), ASB23 (Irvin *et al.*, 1998), HMS2, HMS3, HMS6 and HMS7 (Guérin *et al.*, 1994), HTG4 and HTG6 (Ellegren *et al.*, 1992), HTG7 and HTG10 (Marklund *et al.*, 1994), LEX33 (Shiue *et al.* 1999) and VHL20 (Van Haeringen *et al.*, 1994). The 15 microsatellites were amplified in 3 multiplex reactions as follows: (8plex: AHT4, AHT5, ASB17, ASB23, HMS6, HMS7, HTG4, and VHL20; 4plex: HMS3, ASB2, HTG10, and LEX33; and 3plex: HMS2, HTG6, and HTG7) (Juras and Cothran 2004). Each reaction had a final volume of 12 µL, containing 50 ng of genomic DNA, from 0.07 to 0.8 pmol of primers,1X PCR buffer, 2.5 mM MgCl2, 0.2 mM dNTPs, and 1 UAmpliTaq for the 8plex, while for the 3plex and 5plex, 1U ChoiceTaq was used. For microsatellite amplification, a hot start procedure was used, in which the genomic DNA and primers were combined and heated at 95 °C for5 min. The temperature was then lowered and held at 85°C for 10 min for the addition of the remaining reagents. Thirty-five cycles were as follows: 95 °C for 1 min, either 56 °C (5plex) or 60 °C (for 8plex) for 30 s, and 72 °C for1 min of annealing. The cycling was completed with a final extension at 72 °C for 15 min. The PCR products were separated by electrophoresis on a 6% polyacrylamide gel using the ABI PRISM 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA). The fragment sizes of the microsatellite alleles were determined using the STRand computer software (www.vgl.ucdaviess.edu/STRand).Numerical nomenclature was used for allele size designation in accordance with the International Society for Animal Genetics. Positive and negative controls were used in each reaction.

The number of alleles (Na), the observed heterozygosity (Hobs), the expected heterozygosity (Hexp),the PIC (polymorphic information content) and the probability of exclusion (PE) were estimated using the software Cervus 3.0 (Marshall *et al.* 1998).

RESULTS AND DISCUSSION

The genetic variability detected, estimated by the number of alleles, expected and observed heterozygosity, and PIC is shown in Table 1. This table also shows the power of exclusion of the analysed loci.

The observed heterozygosity values range from 0.484 (HTG7) to 0.935 (VHL20) with the mean expected heterozygosity (He=0.763) slightly higher than the observed (Ho=0.761).

The number of alleles per locus varied from 3 to 12and the mean number of alleles detected was 7.53, a value close to that reported for the Barb horses in Morocco (7.32) (Pablo-Gómez *et al.* 2016) and Algeria (7.64) (Berber *et al.* 2014).

The PIC values are higher for those loci with higher number of alleles, namely ASB17, VHL20 and HTG10, and these loci are the ones showing highest exclusion probabilities. Considering the cumulative value for all 15 loci the probability of paternity exclusion is 99.99%, which allow us to say that this set of microsatellites can be effectively used for paternity tests in the Nigerian horses. All the loci analysed, except HTG7, can be considered highly informative, according to Botstein *et al.* (1980), since their PIC values are higher than 0.500.

The observed heterozygosity is similar to that found for the Moroccan Barb (H=0.766), and the Algerian Barb (H=0.752).

CONCLUSION

To our knowledge this was the first analysis of genetic variation in Nigerian horses. Microsatellites proved to be useful genetic markers for the genetic characterization of these horses. The data and information found here represent a preliminary approach to accomplish the genetic characterization of the Nigerian horses. Therefore further analyses are required in order to better characterize these horses and compare them with other African horse breeds.

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Table 1. Number of alleles (Na), observed heterozygosity (Hobs), expected heterozygosity (Hexp), polymorphic information content (PIC) and exclusion probabilities (PE) for the 15 analyzed loci.

Locus	Na	Hobs	Hexp	PIC	PE
AHT4	8	0.839	0.839	0.802	0.829
AHT5	5	0.710	0.705	0.657	0.665
ASB2	9	0.839	0.787	0.745	0.769
ASB17	12	0.839	0.888	0.861	0.903
ASB23	7	0.806	0.840	0.802	0.824
HMS2	9	0.710	0.796	0.753	0.775
HMS3	8	0.774	0.695	0.656	0.690
HMS6	5	0.613	0.593	0.539	0.528
HMS7	7	0.839	0.775	0.724	0.725
HTG4	7	0.742	0.721	0.680	0.709
HTG6	6	0.774	0.729	0.671	0.659
HTG7	3	0.484	0.579	0.484	0.421
HTG10	10	0.677	0.844	0.812	0.854
LEX33	7	0.838	0.792	0.749	0.769
VHL20	10	0.935	0.865	0.834	0.871
Mean	7.53	0.761	0.763	0.719	
Cumulative					0.9999