A Haplotype Diagnostic for Polled in Australian Beef Cattle

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ABSTRACT: A DNA test to assign poll genotype in Australian beef cattle breeds was released in 2010 and was based on a strong association between an allele (303) at marker CSAFG29 and the polled phenotype in Brahman and other tropical breeds. A pre-commercialisation field trial revealed that this marker was not able to accurately assign poll genotype in a number of other breeds, including Limousin and Brangus, due to the high frequency of another allele (305) that was known to be associated with both polled and horned across a variety of breeds. Using a haplotype test the current study demonstrates that there are two sources of the 305 allele in Limousin and several alleles at CSAFG29 that are associated with polled in Brangus. The haplotype based test has resulted in a much better diagnostic for polled in these breeds.
Keywords: beef cattle; polled; haplotype

Introduction

Dehorning is routinely practiced in beef cattle as horns are an important cause of bruising, hide damage and other injuries, particularly in yards, feedlots and during transport. Although it is advisable to dehorn at a young age, as a result of mustering practices in northern Australia, dehorning is frequently carried out in older calves between 3 and 10 months of age. Dehorning in older calves is labour intensive and causes more pain to the animal. The wound takes longer to heal, is prone to secondary infection and leads to mortality in some cases.

In 2010 the Beef Cooperative Research Centre in Australia released a DNA test for polled. The test was developed to be informative in *Bos indicus* breeds and their crosses, which are the dominant beef genotype in northern Australia, and for which the commercially available diagnostic tests for polled did not cater for. One allele (303 bp in length) at the microsatellite CSAFG29 was strongly associated with the polled phenotype in Brahman cattle; in the Brahman validation population all but 1 of 87 polled animals had at least one copy of the 303 bp allele (Mariasegaram et al., 2012). This marker (CSAFG29) was the basis of a commercial test for polled in Australia from 2010-2013.

A pre-commercialisation trial of the CSAFG29 marker in a range of beef breeds demonstrated the this test was only able to assign poll genotype in some breeds. Limousin, and breeds with high proportions of Angus such as Brangus were particularly problematic, with the test only able to accurately assign poll genotype to 39% and 38% of Limousin and Brangus animals tested, respectively. This was due to the high frequency of another allele in these breeds, 305 bp in length, that was associated with both the polled (P) and horned (H) alleles at the poll locus (Henshall et al., 2011).

It was hypothesised at the time that there were two sources of the 305 allele observed at CSAFG29: one that formed a haplotype with the H allele at the poll locus and which was prevalent in French Limousin, and one that formed a haplotype with the P allele at the poll locus and was primarily derived from the Angus breed (Henshall et al., 2011). Here we describe a 10 locus microsatellite haplotype test that demonstrates two sources of the 305 allele at CSAFG29 and which has resulted in a much better diagnostic for polled in Australian beef breeds.

Materials and Methods

Animals. A total of 1,759 animals were used in this study. The animals were from a wide range of breeds and crosses and had a variety of phenotypes (polled, scurred or horned) as described in Henshall et al., (2014). A range of breeds and crosses covering *Bos taurus* and *Bos indicus* origins were selected for genotyping in order to establish the variability of the markers tested and the number of haplotype alleles that could be expected across a given breed. Animals were selected for the study on the basis of availability of a stored sample of DNA for genotyping, linked to a phenotype record. For 85 Limousin bulls we had progeny test data, and under the assumption that polled is fixed in the Angus breed, we assigned a genotype of PP to all samples from Angus.

Stored DNA was available from animals used in previous research projects and from animals submitted for testing on a commercial basis with the existing CSAFG29 marker test. Where possible, samples within breed were chosen to be balanced across phenotypes, and from a broad spread of the genetics available in Australia for that breed (based on known owner records of the animals submitted for testing).

Markers and genotyping. The ten microsatellite markers used in this study were all discovered in the study by Mariasegaram et. al. (2012) and are described in detail in Henshall et al. (2014).

Samples were a mixture of tail hairs and stored DNA from previous research projects. DNA was extracted from hair follicles using a proteinase-K digest by incubating the sample at 60°C for 45 min and then at 95°C for 45 min. DNA samples utilised from previous research projects had been extracted from semen samples using a standard phenol-chloroform method. PCR was performed in a total volume of 12 µl containing 10-20 ng DNA, 10 µM forward and reverse primers, 0.12 µl Kappa Taq (GeneWorks, Adelaide, Australia) and standard PCR cycling conditions on an Applied Biosystems thermocycler. Capillary fragment separation was performed on an ABI3730 Genetic Analyser and genotypes analysed with GeneMapper

Table 1.

			Count of Haplotype by Breed and Phenotype						
Haplotype	*Count Hap.*	*Association*	*Angus (Polled)*	*Brahman (Horned)*	*Brahman (Polled)*	*Brangus (Horned)*	*Brangus (Polled)*	*Limousin (Horned)*	*Limousin (Polled)*
A-305-AACAAACA	149	Horned	0	0	0	0	0	31	40
A-305-ABAAAEAA	136	Polled	18	0	2	1	3	0	88
A-305-AAAAABAA	103	Polled	34	0	1	0	14	0	17
A-305-AACAAAAA	11	Horned	0	0	0	0	0	1	5
A-305-AAAEGLAA	11	Horned	0	0	0	0	0	5	4
F-305-AACAAACA	8	Horned	0	0	0	0	0	1	6
A-305-AAEAABAA	7	Horned	0	0	0	0	0	2	5
A-305-AAEFAGAA	7	Horned	0	0	0	0	0	2	1
A-305-AACAAAAB	5	Horned	0	0	0	0	0	1	0
A-305-AACAAGCA	5	Horned	0	0	0	0	0	1	0
A-305-AABAAGAA	3	Horned	0	0	0	0	0	1	1
A-305-AAFAAADA	3	Polled	0	0	0	0	0	0	3
A-305-AAAAAKAA	2	Polled	0	0	0	0	0	0	1
A-305-AAODFCCA	2	Horned	0	0	0	0	0	2	0
A-305-IABAAAAA	1	Polled	0	0	0	0	0	0	1
C-305-AACAAPCB	1	Horned	0	0	0	0	0	1	0
H-305-AAFBABAB	1	Polled	0	0	0	0	0	0	1

A list of haplotype alleles carrying the 305 allele at CSAFG29 in the Brangus and Limousin population. For all marker alleles other than the 305 allele at CSAFG29, alleles have been alphabetized to better demonstrate the heterogeneity around the CSAFG29 marker. Each haplotype allele carrying a 305 at CSAFG29 observed in the Limousin and Brangus populations is listed with its association with the polled or horned allele at the poll locus, the number of times the haplotype was observed in the Angus population and the number of times it was observed in the polled and horned Brahman, Brangus and Limousin populations.

(Applied Biosystems) using the Liz-500 as a size standard reference (Applied Biosystems).

Data analysis. The haplo.em function from the haplo.stats package (Sinnwell et al.) in R (R Core Development Team) was used to estimate haplotypes from the diploid genotypes. The haplotype alleles were subsequently assigned as horned or polled based on the phenotype data associated with each sample. Details on the method used to assign the haplotypes are outlined in Henshall et al. (these proceedings). Briefly, haplotypes can be assigned as horned or polled if they are (a) observed in progeny-tested animals (eg. both haplotypes are polled if the animals is progeny-tested homozygous polled); (b) observed in horned animals (both haplotypes are horned); (c) observed in animals that are polled or scurred and one haplotype is known to be horned (the other haplotype must be polled); or (d) observed in animals that are polled and are homozygous for the haplotype (both haplotypes must be polled). If a haplotype has not been observed in one of these situations then it cannot be assigned as horned or polled.

Results and Discussion

Assignment of haplotype alleles to Polled or Horned. A total of 448 haplotype alleles were observed in this population of which around 250 could be assigned as horned and around 60 could be assigned as polled based on the criteria above. A proportion of haplotypes could not be assigned as either horned or polled but these were generally at low frequency in the population so accounted for a small number of animals in the dataset. The frequency of unassigned haplotypes varied from breed to breed and are summarised in detail in Henshall et al. (2014).

There are two clear sources of the 305 allele at CSAFG29 in Limousin. The haplotype test clearly demonstrated that two sources of the 305 allele segregated in the Limousin population. The most common haplotype in our sample of polled Limousin animals is at a frequency of 0.20, is a polled haplotype carrying 305 at CSAFG29 and is commonly seen in Angus. Similarly, another polled haplotype in Limousin that carried a 305 at CSAFG29 was at a frequency of 0.04 in this population and was the most common haplotype in our sample of Angus. These two polled haplotypes accounted for over 20% of haplotypes segregating in the polled Limousin population.

The second most common haplotype in polled Limousin carrying 305 at CSAFG29 was at a frequency of 0.09 and was a horned haplotype. Several other horned haplotypes with a combined frequency of around 5% carried a 305 at CSAFG29.

This data demonstrates why the single marker CSAFG29 test was unable to assign poll genotype in a large proportion of polled Limousin animals. Keeping in mind that only polled animals will be submitted for testing, it would be expected that over 60% of animals would carry at least one copy of 305 at CSAFG29, an allele that has clear associations with both horned and polled in this breed.

The 305 allele at CSAFG29 is a polled allele in Brangus. The second most common polled haplotype allele in Brangus carried a 305 at CSAFG29 and is the most common haplotype allele in our sample of Angus (and the second most common in polled Limousin, Table 2). The most common polled haplotype in Brangus carried a 303 at CSAFG29 (data not shown). There was no cases where a haplotype allele carrying 305 (or 303) at CSAFG29 was

associated with horned allele at the poll locus in the Brangus population in this study, except for one polled haplotype that was seen in a horned Brangus animal. In this case, the evidence is that this haplotype is polled (Table 1) so its presence in a horned animal could be considered a phenotyping error or incomplete penetrance.

Table 2.

		% Informative Result	
Breed	*Number*	*Haplotype*	*Single Marker Test*
Brahman	434	89%	89%
Brangus	115	97%	38%
Limousin	360	95%	39%

Percentages of animals that had polled genotype assigned with the haplotype test and the original single marker test. The results for the haplotype test include commercial samples submitted with phenotypes since the launch of the test in November 2013.

So, why the low rate of assignment of poll genotype in Brangus using the single marker test? Firstly, the polled haplotype carrying 305 at CSAFG29 was at moderate frequency in polled and scurred Brangus (around 0.20). This allele was designated as horned in most breeds and as such, polled Brangus animals carrying this allele would be incorrectly assigned as carrying a horned allele. Secondly, the haplotype test revealed other alleles at CSAFG29 that are associated with polled (which the single marker test would assign as horned) and which account for around 6% of haplotypes in polled Brangus.

Designation of poll genotype in Limousin and Brangus. The haplotype test was much better at assigning poll genotype (PP or PH) in the Limousin and Brangus animals compared with the single marker test based on CSAFG29 alone (Table 2). In Limousin, 95% of animals tested could be assigned a poll genotype (as compared with 38% of animals using the CSAFG29 test). In Brangus, 97% of animals tested could be assigned a poll genotype (as compared with 38% of animals using the CSAFG29 test). In Limousin, the increase in assignment rate can be attributed to the haplotype test being able to distinguish between 305-P and 305-H haplotypes. In Brangus, the increase in assignment rate can be attributed to the haplotype test being better able to attribute horn or poll status across a variety of haplotype alleles that are associated with polled. As more animals with phenotype information are tested and unknown haplotypes are able to be assigned as horned or polled, it is expected that these figures will increase.

Conclusion

The current study demonstrated two clear sources of the 305 allele at CSAFG29 in Australian Limousin cattle, one associated with horned from French Limousin and another associated with polled that was acquired from Angus. The 305 allele at CSAFG29 in Brangus is exclusively polled in our dataset, but the haplotype test revealed other alleles at CSAFG29 that are associated with polled. The haplotype test has resulted in a much better diagnostic for polled in these breeds.

Acknowledgments

This project was supported by Meat & Livestock Australia. We acknowledge the use of samples from the Beef Cooperative Research Centre, and samples and phenotypes collected by many breeders and breed associations across Australia.

Literature Cited

Henshall, JM, Piper, EK, McDonald, CA: Effects of ascertainment bias when testing for polled in Australian beef cattle. *Proc of the Association for the Advancement of Animal Breeding and Genetics* 2011, **19:** 79-82

Henshall, JM, Piper, EK, Tier, B: *Genetics, Selection & Evolution* 2014 (submission imminent)

Henshall, JM, Piper, EK, Tier, B: Haplotype tests for diagnosis of QTL and genes. (These proceedings)

Mariasegaram M, Harrison BE, Bolton JA, Tier B, Henshall JM, Barendse W, Prayaga KC: Fine-mapping the POLL locus in Brahman cattle yields the diagnostic marker CSAFG29. *Animal Genetics* 2012, **43:**683-688

Sinnwell JP, Schaid DJ: haplo.stats: Statistical Analysis of Haplotypes with Traits and Covariates when Linkage Phase is Ambiguous. R package version 1.6.3. edition; 2013

R Core Team: 2013. R: A Language and Environment for Statistical Computing.