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Short communication

Validation of the *POLLED* Celtic variant in South African Bonsmara and Drakensberger beef cattle breeds

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ABSTRACT

An increased awareness of animal welfare necessitates the breeding of genetically polled animals, especially since more than 70% of South African beef cattle are rounded off in commercial feedlots. The Bonsmara and the Drakensberger, two locally developed breeds, play a major role in beef production in South Africa. The causative mutation for polledness in these breeds have not been confirmed, therefore, this study aimed to validate the *POLLED* Celtic variant as the causative mutation of polledness in the South African Bonsmara and Drakensberger beef cattle breeds. A total of 386 animals, consisting of Bonsmara, Drakensberger and Herefords (included as a *Bos taurus* control), were tested for the Celtic mutation by PCR-based screening. Phenotypically polled and scurred animals were found to carry at least one copy of the Celtic allele (P_C) whereas horned animals were homozygous wild type. The highest frequency of homozygous polled animals ($P_C/P_C = 0.337$) was observed in the *Bos taurus* control (Hereford breed) while the majority of the Bonsmara animals were heterozygous polled ($P_C/p = 0.591$). For the Drakensberger, a heterozygous (P_C/p) genotypic frequency of 0.346 was observed, with the majority of animals being horned ($p/p = 0.639$). In the Bonsmara and Hereford breeds, a high proportion of heterozygous polled animals were phenotypically scurred, emphasizing the importance of correct phenotyping at farm level. This research validates the Celtic mutation as causative mutation for polledness in indigenous South African beef cattle breeds. It also demonstrates the current challenges with regards to both phenotypic and genetic verification of the scurs phenotype and requires further investigation in South African beef cattle breeds.

1. Introduction

Since the domestication of cattle, selection practises were focused on adapted animals and aesthetic traits, and horned cattle were favoured by selection. Horns, especially in male animals, were associated with fertility (Knierim et.al., 2015; Schafberg and Swalve, 2015). However, over the past few decades the focus has shifted towards sustainable animal production, with an increased awareness of animal welfare. Horns in cattle are a major cause of bruising, hide and carcass damage, as well as other injuries, but the practice of dehorning cattle has serious welfare implications (Graff and Senn, 1999; Windig,et.al., 2015). Breeding genetically polled animals would provide a long-term solution and welfare friendly alternative to dehorning.

The *POLLED* locus has been mapped to the centromeric region of BTA1 in a number of cattle breeds (Drögemüller et al., 2005; Georges et.al., 1993; Seichter et al., 2012) and three distinct causative variants have recently been identified at this locus, namely the Celtic, Friesian and Mongolian alleles (Allais-Bonnet et al., 2013; Medugorac et al., 2012, 2017). The Celtic allele (P_C) is responsible for polledness in most of the European *Bos taurus* breeds, while the Friesian allele (P_F) predominantly governs the polled phenotype in the Holstein Friesian breed (Medugorac et al., 2012). The Mongolian allele (P_M) has been described only in East Asian *Bos taurus* and *Bos grunniens* breeds (Medugorac et al., 2017). None of these mutations are located in known coding or regulatory regions, thus adding to the complexity of the molecular basis of polledness. The genetic basis of polledness is further complicated by the presence of scurs, which develop as small horn-like growths in the same area as horns on the skull, but these abnormal horns are loosely attached to the skull (Capitan et al., 2009). The *POLLED* locus has been found to be epistatic to scurs in both sexes.

In South Africa, the red meat industry plays a major role in livestock production, with more than 70% of all beef cattle slaughtered in the

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formal sector originating from commercial feedlots (Scholtz et al., 2008). In Bonsmara herds, the polled trait occurred either spontaneously due to the Shorthorn/Hereford ancestors from which the breed was developed or by infusion through the upgrading of Red Poll and Red Angus cows to Bonsmara stud status (Schmulian, 2006). The Drakensberger breed is naturally horned, with the assumption that polledness was introgressed in the breed by upgrading with naturally polled breeds, such as the Black Angus. In South Africa, polledness was historically not a trait selected for by beef cattle breeders, mainly due to the belief that polled animals are inferior compared to horned animals (Schmulian, 2006). However, over the past two decades, South African breeders realized the advantages of polled cattle and showed an increased interest in breeding polled animals, primarily due to welfare and market preferences.

The majority of previous research on the *POLLED* locus and polledness has been performed in European breeds (Allais-Bonnet et al., 2013), and South African indigenous breeds are genetically distinct from the European *Bos taurus* breeds (Makina et al., 2014). Besides the two main types of cattle, *Bos taurus* and *Bos indicus*, indigenous African cattle, such as the Sanga, are also found in South Africa. The Drakensberger is classified as a Sanga breed, which are a hybrid between *Bos taurus* and *Bos indicus* (Rege and Tawah, 1999).

A preliminary study on polledness in Bonsmara cattle indicated association between the polled phenotype and nine microsatellite markers on BTA1 (Schmulian, 2006). The causative mutation for polledness is still unknown for indigenous South African cattle. This study forms part of a larger research project to investigate the inheritance patterns of the *POLLED* and *SCURS* loci in indigenous South African beef cattle breeds. This study investigated polledness in the South African Bonsmara and Drakensberger beef cattle breeds, with the aim of validating the Celtic variant as the causative mutation of polledness in these breeds.

2. Materials and methods

2.1. Animals and phenotypes

The study was performed with consent from the respective Breeders' Associations, as well as individual breeder consent, and ethical approval from the University of Pretoria (EC170424-110). Hair samples and phenotypic records of the horn status of mature registered purebred animals were provided by four Bonsmara breeders, six Drakensberger breeders and three Hereford breeders. The samples included animals with polled, horned and scurred phenotypes. Samples with an unknown phenotype and sex were excluded from analyses and a total of 386 animals were included in this study, consisting of 164 Bonsmara and 133 Drakensberger. 89 Hereford were included as a *Bos taurus* control.

2.2. Celtic genotyping

Genomic DNA were extracted from the hair samples with a Zymogen Tissue kit (www.zymoresearch.com) in the Animal Breeding and Genetics laboratory at the Department of Animal and Wildlife Sciences, University of Pretoria. The polled, horned and scurred animals were screened for their status for the Celtic mutation at the *POLLED* locus using a microsatellite marker-based diagnostic test. To identify the Celtic mutation, the CELT primer (CELT-Fw: GAAGTGTG GCCGGTAGAAAA and CELT-Rv: ATCAAGGACACCTCCCACAC) was used (Allais-Bonnet et al., 2013). This screening allows the identification of carriers of the Celtic mutation, as well as the identification of genotypic status (P_C/P_C, P_C/p or p/p).

The PCR reaction was performed with a final volume of 15 µl. The amplification reaction contained 8 µl Bioline MyTaq Red Mix® enzyme (www.bioline.com), 1.4 µl molecular grade water, 0.3 µl each of both forward and reverse primer [10 pmol/µl] and 5 µl genomic bovine DNA. The PCR conditions were performed as follow: 94 °C for 5 min, 39 cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s, with a final extension step of 72 °C for 5 min. The PCR products were visualized on a 3% agarose gel with a 100 bp size ladder to determine the fragment size of the products. There is a 202 bp difference between the Celtic and wildtype allele.

2.3. Statistical analysis

Genotype frequencies were calculated for the three possible genotypes of the Celtic allele (P_C/P_C, P_C/p and p/p) by direct counting. A Hardy–Weinberg Equilibrium (HWE) *p*-value was calculated for the genotype frequencies using a Chi-square test and the significance threshold was set at 0.05. Pearson correlation coefficients between the phenotypes recorded on farm and the Celtic genotypes obtained from the PCR-based Celtic screening of the samples, were calculated by R software v3.3.1 (R Core Team, 2013). Correlation coefficients were calculated to validate the accuracy of the Celtic allele to indicate the polled status of an animal, as well as to determine the accuracy of the phenotypic recording of each sample group. The Pearson correlation (r) measures a linear dependence between two variables, x and y, where in this case x is equal to the on farm recorded phenotype of the horn status of each animal, and y equals the observed Celtic genotype obtained from the PCR-based screening.

3. Results and discussion

The South African Bonsmara, Drakensberger and Hereford beef cattle breeds were screened for the Celtic allele (P_C) (Medugorac et al., 2012) and both homozygous and heterozygous polled animals were observed. It was possible to distinguish between horned, homozygous polled and heterozygous polled animals at a genotypic level.

The frequency of the observed genotypes for the Celtic allele in the three breeds are shown in Table 1. All the phenotypically polled and scurred animals were found to carry at least one copy of the Celtic allele (P_C) whereas horned animals were homozygous wild type. Based on the HWE *p*-values ($p < 0.0001$, Table 1), the genotypic frequencies observed were significantly different and deviated from Hardy–Weinberg Equilibrium ($p < 0.0001$). The deviation from HWE was expected, due to indirect selection for the *POLLED* locus in these breeds. The Hereford had the highest frequency of homozygous polled animals ($P_C/P_C = 0.337$), as expected due to the selection preference for polled Hereford in South Africa. In the Bonsmara the majority of the animals tested

Table 1
The total observed genotypes and genotypic frequencies for the Celtic variant observed in three South African beef cattle breeds.

Breed	Genotype P_C/P_C Total	Frequency	P_C/p Total	Frequency	p/p Total	Frequency	Total	HWE*p-value
Bonsmara	29	0.177	97	0.591	38	0.232	164	< 0.0001
Drakensberger	2	0.015	46	0.346	85	0.639	133	< 0.0001
Hereford	30	0.337	46	0.517	13	0.146	89	< 0.0001
Total	61		189		136		386	

* HWE = Hardy–Weinberg equilibrium