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.

Keywords: Polledness, Heterozygous polled, Sanga cattle, Scurs

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 (Georges et al., 1993; Drögemüller et al., 2005; Seichter et al., 2012) and three distinct causative variants have recently been identified at this locus, namely the Celtic, Friesian and Mongolian alleles (Medugorac et al., 2012; Allais-Bonnet et al., 2013; Medugorac et al., 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 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: GAAGTGTGGCCGGTAGAAAA 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 30s, 55 °C for 30s and 72 °C for 30s, with a final extension step of 72 °C for 5 min. The PCR products were visualized on a 3% agarose gel with a 100bp 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 were heterozygous (P_C/p = 0.591) for the polled phenotype, with a lower frequency of homozygous polled animals (P_C/P_C = 0.177). The majority of phenotypic polled Drakensberger animals were heterozygous polled (P_C/p = 0.346) with very few animals being homozygous polled (P_C/P_C = 0.015). The lower frequency of the polled allele in the Drakensberger can be attributed to the fact that this breed is historically horned and very few breeders have introgressed the polled allele into their breeding herds. Some Drakensberger breeders also are of the opinion that polled Drakensberger bulls are inferior compared to horned bulls, with polled bulls exhibiting lower masculinity and a higher incidence of penile prolapses (Pers. Comm., D. Orsmond, May 2017). In general, cattle breeders historically showed a preference for horned bulls due to a perception that horned animals were more fertile and exhibited stronger male characteristics. This perception may have led to selection preference for horned bulls in a number of breeds (Götz et al., 2015).

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

Breed	**Genotype**						**Total**	**HWE* p-value**
	Pc/Pc		**Pc/p**		**p/p**			
	Total	**Frequency**	**Total**	**Frequency**	**Total**	**Frequency**		
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

It can be concluded that the polled phenotype in the South African Bonsmara, Drakensberger and Hereford are genetically determined by the Celtic allele (P_C). This corresponds to the ancestry of the breeds, as introgression and upgrading with *Bos taurus* breeds occurred in both Bonsmara and Drakensberger breeds, and the Hereford is an exotic *Bos taurus* breed. The Celtic allele have been identified in most European cattle *Bos taurus* breeds (Allais-Bonnet et al., 2013) and more recently in a synthetic Chinese cattle breed, Shuxuan, which was crossbred with both Simmental and Holstein semen. Chen et al. (2017) observed the Celtic allele in the Shuxuan cattle at a frequency of 0.437.

The *SCURS* locus is epistatic to the *POLLED* locus and according to the model of Long and Gregory (1978), a sex-influenced expression pattern is assumed for the scurs phenotype and in males, the heterozygote (*Scsc*) is usually scurred, while in females only the homozygote (*ScSc*) is scurred. Furthermore, males that are heterozygous for scurs must also be heterozygous at the *POLLED* locus in order for the scurs phenotype to be expressed. Thus, animals with a scurs phenotype should show a heterozygous polled genotype (Long and Gregory, 1978) for the Celtic allele (P_C/p), which further suggests that animals with a heterozygous polled genotype can either be phenotypically polled or scurred. However, scurs cannot be identified on a genotypic level based on the Celtic allele (P_C). In all three breeds phenotypically scurred animals were genotyped as heterozygous polled carriers of the P_C allele. The proportion of animals with a heterozygous polled genotype (P_C/p) for the Celtic mutation, that are phenotypically identified as scurred are shown in Table 2. In the Bonsmara breed, 50% of the male heterozygous genotypes (P_C/p) corresponded with a scurred phenotype, while in the Hereford breed, 42% of female animals that were genotyped as heterozygous polled (P_C/p) were phenotypically scurred. None of the homozygous P_C/P_C animals have a scurred phenotype and therefore the effect of the P_C allele is additive.

Table 2 The horn status phenotypes for each breed as recorded on farm per sex, with the Celtic genotype observed for each breed per phenotype for male and female animals (the corresponding genotypic frequencies are indicated in brackets)

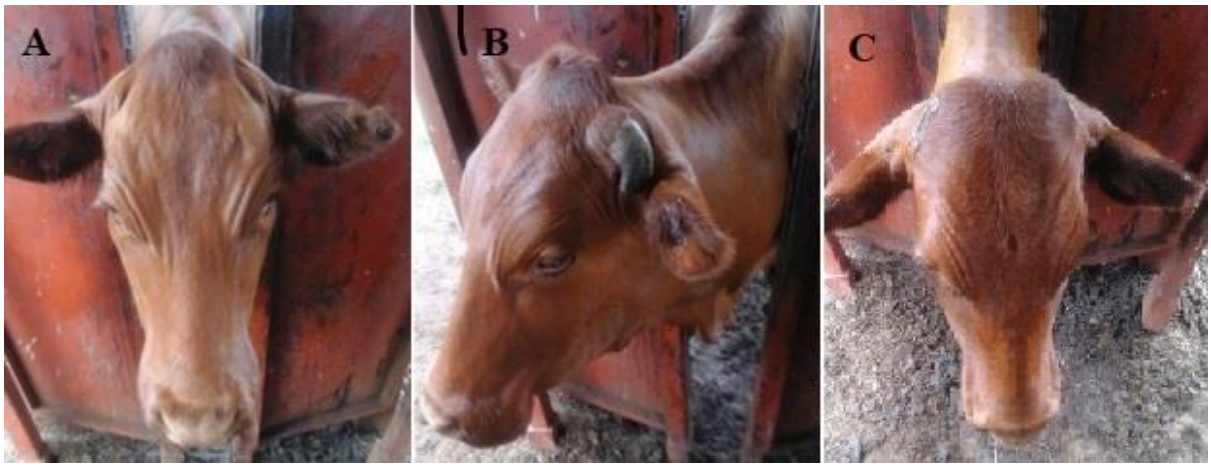
Breed	Sex	On farm phenotype				Genotype*				
		Polled	Scurred	Horned	Total	Pc/Pc Polled	Pc/p polled	Pc/p Scurs	p/p horned	Total
Bonsmara	Male	38	27	8	73	14 (0.192)	24 (0.329)	24 (0.329)	11 (0.151)	73
	Female	59	13	19	91	15 (0.165)	43 (0.473)	6 (0.066)	27 (0.297)	91
Drakensberger	Male	36	2	9	47	1 (0.021)	25 (0.532)	2 (0.043)	19 (0.404)	47
	Female	49	0	37	86	1 (0.012)	19 (0.221)	0 (0.000)	66 (0.767)	86
Hereford	Male	47	11	5	63	27 (0.429)	21 (0.333)	9 (0.143)	6 (0.095)	63
	Female	8	15	3	26	3 (0.115)	5 (0.192)	11 (0.423)	7 (0.269)	26

* P_C – dominant polled allele for the Celtic variant, p – recessive horned allele

Correlation coefficients calculated to determine the accuracy of phenotypic recording amongst the breeds and the results of the Celtic PCR-based screening, indicated that there is a strong positive correlation between the phenotypes of the horn status of animals identified on farm level and the Celtic genotype. The high positive correlation in the Bonsmara breed (0.84; $p < 0.001$), indicates more accurate phenotypic recording of the polled status in the Bonsmara herds included in this study. The low and moderate correlation coefficients for the Drakensberger (0.55; $p < 0.001$) and Hereford (0.62; $p < 0.001$) breeds, respectively, can be attributed to the occurrence of a few discrepancies between the phenotype identified and the genotype obtained from the Celtic screening. These discrepancies can be explained by incorrect phenotypic identification of animals, since the inconsistencies were found between horned and scurred phenotypes for the Hereford breed. In the Drakensberger herds, animals were even incorrectly phenotyped as polled. Table 2 further demonstrates the poor phenotypic recording of some animals, by indicating the differences in the on farm recorded phenotype and the actual genotype that were observed.

These inconsistencies emphasize the importance of visual inspection of animals both at a young age, as well as between 18 to 24 months of age. It is also important that farmers and farm workers be trained to be able to distinguish the difference between a horned and scurs phenotype. Polledness is an observable phenotype that can be identified at a relatively young age and which does not change with age. Scurs develop as small horn-like growths in the same area as horns on the skull, but these protuberances are loosely attached to the skull (Capitan et al., 2009). The scurs phenotype, however, develops approximately after four months of age and needs to be confirmed between 18 and 24 months of age (Capitan et al., 2009). The different phenotypes, with regards to the horn status in Bonsmara cattle observed in this study, are illustrated in Figure 1. It can be clearly seen that there is a marked difference in the head shape of the polled versus dehorned animals

Figure 1 The polled (A), scurs (B) and dehorned (C) phenotype in Pc/p polled, Pc/p scurred and p/p horned South African Bonsmara cows, respectively.



* A horned animal was not included, since all animals must be dehorned according to the Bonsmara breed standard.

It is standard practice to dehorn cattle at a young age by means of physical dehorning, but in most cases without the appropriate pain relief (Knierim et al., 2015). The practice of dehorning has increasingly become a welfare concern and alternatives to dehorning are advocated worldwide. Breeding genetically polled cattle is a long-term, non-invasive and welfare friendly alternative to dehorning. Identification of genetically polled animals through a diagnostic test would therefore be advantageous, but a specific commercial diagnostic test for the polled phenotype is not currently available in South Africa. The DNA tests that are available internationally are applicable to European *Bos taurus* breeds, which can give inconclusive results for indigenous South African and Sanga cattle breeds. Developing a commercial diagnostic test in South Africa for indigenous cattle breeds based on the Celtic mutation will contribute to accurate testing in these breeds, that will enable breeders to market certified polled bulls

4. Conclusion

PCR-based screening of the Celtic mutation concluded that the polled phenotype in the South African Bonsmara, Drakensberger and Hereford are genetically determined by the Celtic allele (P_C). Therefore, the *POLLED* Celtic variant is validated as the causative mutation of polledness in three South African beef cattle breeds and can be used as an efficient diagnostic test for polledness. This study highlighted the current limitations of accurate phenotypic recording of the horn status. Current limitations include the difficulty in recording scurs accurately, due to the development of scurs, indiscriminate dehorning and extensive farming

systems that make phenotypic recording difficult. It also confirmed that scurs cannot be identified on a genotypic level with the Celtic screening, and the *SCURS* locus requires further investigation in Sanga beef cattle breeds.

Conflict of interest

All authors declare that they do not have any actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, the current work.

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