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Polledness in Argentinean Creole cattle, five centuries surviving

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Summary

Polledness has been shown to have autosomal Mendelian inheritance, with the polled locus being dominant to the horned locus. This trait was mapped to the BTA1 centromeric end in several breeds. One of the distinctive attributes of Creole cattle, such as the Argentinean Creole, is the presence of long, lyre-shaped horns. However, polled native animals were reported before the introduction of modern selected European breeds. Here, we studied the origin of the polled mutation, either independent or introgressed, in a Creole line from the Creole cattle founder group at the IIACS-INTA Leales Experimental Station (northwest Argentina). The study sample (65 animals: 26 horned and 39 polled) was genotyped using high-density SNP microarrays and three previously reported genetic markers (P_{202ID}, P_{80kbID} and P_G). A genome-wide association study, selection signatures, linkage disequilibrium analysis and copy number variations were used to detect the responsible region and the segregating haplotypes/alleles. The interval mapped in the Leales herd (1.23–2.13 Mb) overlapped with the region previously reported in several European cattle breeds, suggesting that the same locus could be segregating in this population. The previously reported variants P_F and P_G were not detected, thus dismissing the Holstein-Friesian and Nellore origins of the polled phenotype in this native breed. Conversely, the presence of the Celtic variant P_C suggests an almost complete co-segregation. The cluster analysis rejected the hypothesis of recent introgression, which is compatible with the historical record of polled Creole cattle in northwest Argentina.

Keywords bovine, genome-wide association study, microarray, native breed, polled, selective sweeps

Polledness was originally defined by Bateson & Saunders (1902) as having autosomal Mendelian inheritance, with polled being dominant to horned (https://omia.org/OMIA000483/9913/). This locus was mapped to chromosome BTA1 (1q12–14) within the centromeric end (Schmutz *et al.* 1995). In the last decade, several authors reported two mutations as being responsible for polledness in the taurine European cattle (Medugorac *et al.* 2012; Allais-Bonnet *et al.* 2013; Rothammer *et al.* 2014; Wiedemar *et al.* 2014; Carlson *et al.* 2016): an 80-kb duplication observed in Holstein-Friesian cattle (UMD3.1 genome build BTA1:g.1909352_1989480dup), called P_F, and a Celtic-origin duplication of 212 bp replacing a segment of 10 bp (UMD3.1 genome build BTA1:g.1706051_1706060delins, g.1705834_1706045dup), called P_c, both widespread in several European breeds. A third polled allele, discovered by Medugorac *et al.* (2017) in Asian cattle, comprises a complex 219-bp duplication–insertion (P_M), also duplicated in P_F. Moreover, the probable existence of additional variants was suggested by Chen *et al.* (2017) in Chinese cattle and by Stafuzza *et al.* (2018) and Utsunomiya *et al.* (2019) in Nellore. In this sense, Utsunomiya *et al.* (2019) reported a 110-kb duplication variant of the polled locus in the Nellore breed (Guarani polled allele; P_G). Interestingly, the reported variants did not occur in coding sequences, a splice site or any known regulatory regions.

Cattle were first introduced into America by the Spanish and spread from the Caribbean islands to the colonies in America (Wilkins 1984; Primo 1992; Felius 1995).

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Simultaneously, there were direct shipments of Portuguese cattle to Brazil (De Alba 1978; Primo 1992). Today, Creole cattle breeds remain in almost all American countries () and are recognized by their longevity, fertility and hardiness (Flori *et al.* 2012). Most of them have retained the long lyre-shaped horns characteristic of Iberian cattle (Giovambattista & Ripoli 2002). However, the existence of polled native animals has been reported since 1770 within Jesuit farms in the current Argentine territory (de Azara 1850), before the introduction of modern selected European breeds. Furthermore, there are some Creole polled breeds, such as Romosinuano and Caracu Mocho, that had been selected toward this phenotype (https://razasbovinasdecolombia.weebly.com/romosinuano.html, http://www.caracumocho.com.br).

By 1950, a Creole herd had been established at the Experimental Station of the Instituto de Investigación Animal del Chaco Semiárido (IIACS-INTA) in Leales, Tucumán, Argentina. The herd was made up of animals phenotypically corresponding with Creole characteristics, geographically isolated and known to have no crossbreeding (Holgado & Rabasa 2001). The founder group included two polled females that were the origin of the actual polled animals. Until now, there has been no report as to whether the polledness in Creole populations is the result of independent mutation(s) or introgression from European cattle (e.g. Angus, Red Poll, Holstein).

To study the origin of the polled mutation in the Leales Creole cattle, 65 animals (39 polled and 26 lyred horned) were genotyped using the Axiom™ Bos 1 Genotyping Array r3 (Affymetrix), and a genome-wide association study (GWAS) was performed using Fisher's exact test and dominant gene action models implemented in PLINK 1.09 software (Purcell *et al.* 2007), considering a case–control phenotype model. The datasets used for the GWAS and admixture analysis were upload to the NAGRP Community File Sharing Platform (www.animalgenome.org), and four previously associated mutations were also genotyped: IFNGR2-intron 3 SNP alleles *AC000158*:g.1 390 292G>A, P_C indel and P_F and P_G duplications (Table S1). Additionally, genotypes of 594 cattle from 11 breeds were available for further analyses (Table S2). The REHH package for R (Gautier & Vitalis 2012) was used to identify signatures of selection between horned and polled Creole groups, and STRUCTURE 2.3.4 (Pritchard *et al.* 2000) was used to detect the possible introgression of the polled allele from other breeds. Finally, linkage blocks (haplotypes) within the significant region were constructed (Gabriel *et al.* 2002; Barrett *et al.* 2005). Details are presented in Appendix S1.

The GWAS was performed using all Leales animals through a case–control model with 339 112 filtered SNPs spanning the 29 autosomal chromosomes. Fisher's exact test revealed 53 significantly associated SNPs ($P < 10^{-8}$), all located on BTA1 (Figs. 1a & S1, Table S3). Of these, 41 SNPs were located at 1.23–4.64 Mb on BTA1, with the peak between 1.23 and 2.13 Mb (13 SNPs in complete linkage disequilibrium, $P < 3 \times 10^{-11}$). When the dominant model was applied, 47 SNPs were significant ($P < 10^{-8}$), 36 of them located between 1.23 and 3.80 Mb on BTA1 (Fig. S1b, Table S3). Additionally, the 13 most highly significant SNPs in the peak ($P < 4 \times 10^{-15}$) coincided with those obtained before between 1.23 and 2.13 Mb. These results suggest no differential selection between the two groups and agree with the proposed autosomal dominant Mendelian inheritance for this trait (http://omia.org/OMIA000483/9913/). Previous works mapped the polled locus to a 381-kb interval (BTA1:1.668–2.049 Mb) in several European cattle breeds (Medugorac *et al.* 2012; Seichter *et al.* 2012) and to a 3.11-Mb region (BTA1: 878–3987 Mp) in Zebuine Nellore cattle breeds (Stafuzza *et al.* 2018), overlapping the interval observed in the present work, and therefore suggesting that the same locus could be segregating in the Leales Creole population.

Considering that both groups belonged to the same population, were mated alternatively and bred together, the only difference being the horned/polled trait, selection signatures should be present only in the region where this trait was encoded. The selection signature analysis across chromosome 1 was estimated through Rsb statistics. As expected, Rsb results showed only a significant peak [$-\log_{10}$ (*P*-value) > 6] at the centromeric end of this chromosome (Fig. S1c). This result agrees with the GWAS and gives additional support to the location of the polled/horned locus within this region in the Leales Creole herd.

Once the BTA1 centromeric region association was confirmed with the polled phenotype in this herd, an ancestral mutation or introgression was tested. The further analysis consisted of genotyping the previously associated mutations—IFNGR2-intron 3 SNP, P_C, P_F and P_G—that were associated in Celtic linage cattle, Holstein-Friesian and Nellore respectively (Medugorac *et al.* 2012; Allais-Bonnet *et al.* 2013; Rothammer *et al.* 2014; Wiedemar *et al.* 2014; Utsunomiya *et al.* 2019). Results of a copy number variation analysis for P_F evidenced that the duplication was absent in all the Leales animals, both polled and horned (Fig. S2). On the other hand, the IFNGR2-intron 3 SNP, previously associated in Holstein, showed no variation, all animals being homozygous (G/G). Considering both results, the Holstein-Friesian origin of the polled phenotype in this breed could be dismissed. The third marker analyzed was the Celtic-origin variant P_C, associated with polledness in many European breeds (Medugorac *et al.* 2012), its effect confirmed by gene editing of horned bovine embryo fibroblasts (Carlson *et al.* 2016). Results showed an almost complete linkage of this mutation with polledness (Fig. S3); polled animals were either homozygous (6.45%) or heterozygous (83.87%) for the duplication, whereas three polled animals were homozygous for the wild type. Interestingly, those animals descended from a common ancestor

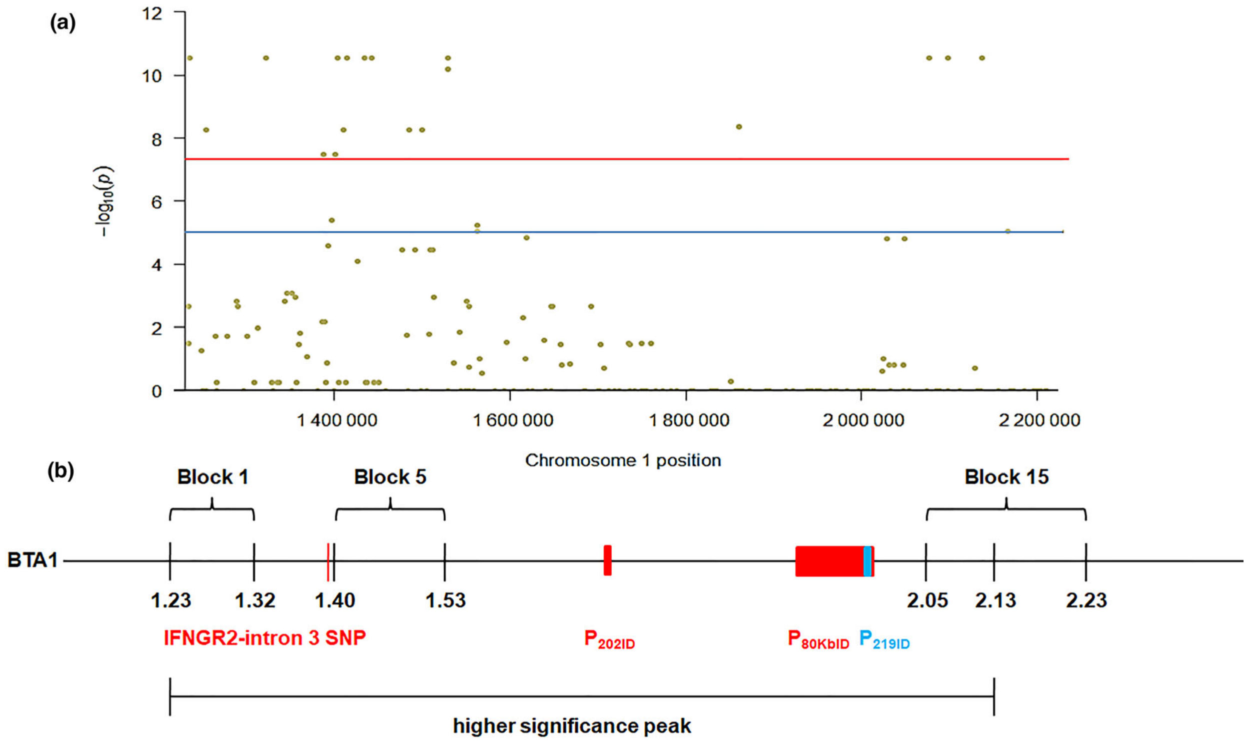

Figure 1 (a) Manhattan plot of a genome-wide association study comparing horned vs. polled Argentinean Creole cattle using a case–control model and Fisher's exact test. The *x*-axis denotes the highly BTA1-associated region (BTA1:1.23–2.23 Mb) with SNP positions plotted in increasing genomic order. The *y*-axis plots the −log10 *P*-value as determined in an association analysis using PLINK 1.09 software (Purcell *et al*. 2007). (b) Diagram of the associated region from the GWAS showing the location of the three linkage disequilibrium blocks, the analysed reported markers and the most highly associated peak.

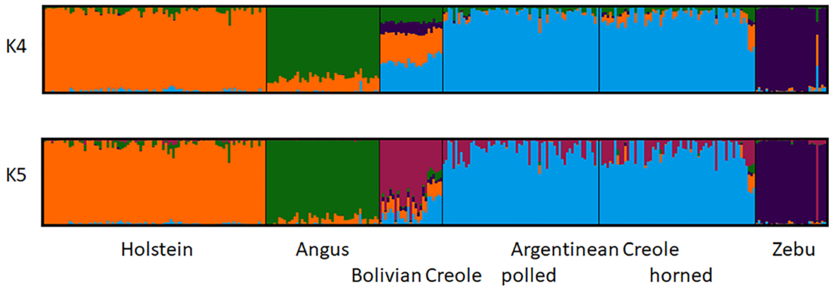

Figure 2 Genome-wide composition analysis of the Leales population of Argentinean Creole cattle, dividing horned vs. polled groups. The Holstein, Angus (blue), Argentinean Creole (red), Bolivian Creole, and Zebu (Brahman and Nellore) genetic fractions are shown for each individual within those breeds. Values were estimated from a subset of SNP data (10K panel) using STRUCTURE software ($K = 5$ and $K = 6$).

born in 1991. These results support the hypothesis of the Celtic origin of this trait in the Leales herd, although resequencing of the associated region in those three animals is required to determine the presence of a possible additional causal mutation.

Despite the availability of registries with the complete genealogy of all animals born in the herd since 1959, a clustering test was performed using STRUCTURE to test for an introgressed origin of the polled mutation. The test included Holstein and Angus as possible sources of the polled allele, Zebu to test the common situation of admixture with Zebuine cattle in sub-tropical regions and Bolivian Creole as another Creole reference. The results suggested no evidence of admixture with the tested breeds and no genetic structure within the Leales herd (Fig. 2). Furthermore, the linkage disequilibrium (LD) analysis did not show conserved blocks around P_C among the breeds analyzed (Fig. S4). These results support the hypothesis that the mutation could have been segregating since the Spanish Colonial time.

To refine the mapping, an LD analysis was performed within the 3.45-Mb associated interval (BTA1:1.23–4.68 Mb) including 437 markers. This analysis revealed

70 LD blocks, 30 of which exhibited $P < 10^{-4}$. Assuming a dominant inheritance of the polled phenotype, it was expected that blocks containing the polled allele would be absent in horned animals and present in homozygote- or heterozygote-status polled cattle. Only three blocks—located between 1.23 and 1.32 Mb, 1.40 and 1.53 Mb and 2.05 and 2.23 Mb and with *P*-values below 10^{-8}—matched these criteria (Table S4). As expected, the highest significance peak obtained from the previous individual SNP case–control analysis (at BTA1:1.23–2.13 Mb) contained these LD blocks (Fig. 1). However, the highest significant peak also contained the reported markers IFNGR2-intron 3 SNP (BTA1:1 390 292), P_C (BTA1:1 705 834–1 706 045) and P_F (BTA1:1 909 352–1 989 480), none of which were located within the three blocks mentioned above (Fig. 1b). The genes contained in these LD blocks are detailed Appendix S2.

In conclusion, the present results support the Celtic origin of the polled phenotype in the studied Creole cattle herd and that another segregating new mutation could be present. Additionally, the absence of recent genetic introgression from other polled breeds raised in the same geographical region would support the hypothesis of an ancient origin of the causal mutation, in agreement with historical data reporting the presence of polled animals at least two centuries ago. The ancient Celtic variant has been detected in independently evolved breeds, such as Nordic, British and Icelandic, allowing this variant to be dated back to at least a millennium ago (Allais-Bonnet *et al.* 2013). Considering the Iberian origin of Latin American Creole cattle, this would widen the geographical distribution of the Celtic variant.

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Ethics statement

Experiments reported in this work comply with the ethical guidelines of the Institutional Committee for the Care and use of Laboratory Animals (CICUAL) from the School of Veterinary Sciences of the National University of La Plata (Buenos Aires, Argentina).

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1 Manhattan plot of a genome-wide association study comparing horned vs polled Argentinean Creole cattle using (a) a case–control model and Fisher's exact test and (b) the dominant model (b). (c) Selection signatures over the 29 autosomal chromosomes between horned and polled Creole populations detected through the LD-based methods Rsb [$-\log10\ (1-\Phi(\text{Rsb})]$ using the rehh package for R software (Gautier & Vitalis).

Figure S2 CNV results for BTA1:1.23–4.64 Mb associated region for polled animals from the Leales herd.

Figure S3 PCR results for P_C variant for polled (P) and horned (H) animals from the Leales herd.

Figure S4 Linkage disequilibrium results using the 'four gamete' method implemented in HAPLOVIEW software within each breed.

Table S1 Primers used for genotyping the polled candidate causal mutations: IFNGR2-intron 3 SNP and P_C.

Table S2 Details of cattle used in the genetic analysis of the polled/horned phenotype.

Table S3 Significant results ($P < 10^{-8}$) of GWAS analysis performed in the Argentinean Leales Creole cattle population.

Table S4 The most highly associated haplotype blocks on BTA1 for the polled/horned phenotype were identified and given a Block ID.

Appendix S1 Material and methods description.

Appendix S2 Genes in the candidate regions.