

## Polled Bali Cattle and Potentials for the Development of Breeding Industry in Indonesia

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### Abstract

A polled Bali Cattle, or a naturally hornless cattle breed was firstly recognized in the early 1980s in Sidendreng-Rappang (Sidrap), South Sulawesi, where Bali cattle (*Bossondaicus*) herd were mixreared with some Brahman cross (BX). Whereas BX is the result of a cross between a Brahman cattle and the hereford or shorthorn (*Bos Taurus*) breed on a commercial ranch in the region. As such a variant is considered as having high productivity trait advantages and/or as even possibly becoming a superior cattle breed of choice being able to develop by the University of Hasanuddin, whereas various researches had been carried out since 1985. To date, the research works had resulted in some findings, however there might have only been up to preliminary stages so far. Vast and thorough investigations on productivity traits of the polled Bali cattle (PBC) variants are being sought. Currently, a science and technology-based local cattle breeding industry development program at the Maiwa breeding centre, Faculty of Animal Husbandry, Hasanuddin University has been carrying out serial studies on genetic profiles of the variants, and also on their growth control and adaptation genes. The genes have been targeted for use as marker assisted selection (MAS).

**Keywords:** Polled Bali Cattle, genetic, breeding industry

### INTRODUCTION

A modern livestock industry either beef or dairy types, when rearing those animals with naturally horned breeds, had mostly been experiencing in substantial economic losses due through animal injuries during transportation, and also at housing or during feedlotting and meat processing at slaughters. As such types of horned animals may also pose a danger to handlers. For these particular reasons, markets are placing more increasing pressure on producers to result in cattle without sharp horns. The pressure is particularly strong from the live export and feedlotting trades. These furthermore, may mean that cattle producers must either to procure them horned less either through a dehorning practice or rearing genotypically polled cattle variants. Otherwise, they face ever-increasing penalties when selling horned cattle. In the worst-case scenario, the beef cattle producers may not be able to sell their horned stocks anymore.

Bruising and carcass damages are major sources of financial losses to slaughter houses in the United States, of which was estimated approximately up to \$46 million per annum. Whereas, studies conducted in Australia have also indicated that cattle horns are proved to be the major

causes of carcass bruising. The type of injury had been shown to be almost double in groups of horned cattle when compared with groups of polled cattle (Mason, 1974; Meischke *et al.* 1974; Prayaga, 2007).

Dehorning or disbudding practices had been used and considered as being one of common farm management practices to get hornless animals, but lately along with an increasing interest in animal welfare, which had placed many livestock production practices under enhanced scrutiny. Such a practice is now categorized as one deed of cruelty to animals (AVMA, 2014). As proofed in beef cattle breed, polledness is a trait of increasingly importance to beef producers for a multitude of reasons. While animal welfare issues attached to dehorning are a key driver for some producers involved with horned breeds to select for polled genetics, many are discovering the added benefits such as reduced labour costs and increased workplace health and safety.

Bali cattle (*Bossondaicus*) are native Indonesian cattle (Baco *et al.*, 2019), which had been domesticated from an indigenous wild type ancestor, Banteng (*Bos javanicus*), since around 3500 BC (Mohammad *et al.*, 2011; Baco *et al.*, 2000). Even though there had been through for a quite long period of domestication, the animal appeared to be still very hardy, of which is able to jump high with a potentially damaging kick (Moekti 2020, unpublished data). A pair of sharp horns in a Bali cattle may even be much more dangerous to human to handle. According to a report from the Bureau of Labour Statistics in the United States of America (USA) during a period 1992 to 1997, there were more than 75,000 workers received injuries and 375 of them were killed from animal related injuries. Whereas, cattle were the most responsible for most cases caused the most injuries caused by farm animals.

There are however several advantages in Bali cattle breed productivity in comparison to other breeds, as they have known to be highly adaptable to harsh environmental conditions, such as critical lands (Murtidjo, 1990; Saputra, 2008; Baco, 2011). The breed had been known to yield a high percentage of meat per carcass weight and also had been proved to have less fat in their meat (Ngadiyono, 1997). It is therefore an attempt to procure a polled Bali cattle (PBC) variant will bring about a promising impact for the improvement of national wide beef cattle industry.

## **A PHENOTYPIC EXPRESSION OF POLLED BALI CATTLE AND INITIATED PRELIMINARY STUDIES**

A variant of polled Bali cattle, or a naturally hornless cattle breed was initially recognized in the early 1980s in Sidendreng-Rappang (Sidrap), south Sulawesi, where mixed breeds Bali cattle (*Bossondaicus*) and Brahman cross (BX). Whereas BX is the result of a cross between a Brahman cattle and the hereford or shorthorn (*Bos Taurus*) breed used as a commercial ranch in the region, known as Bila United LiveStock. In a period over 1985-1986, the University of Hasanuddin established a miniranch in Pattalassang, Gowa, where a preliminary study on PBC was initiated. A few PBC males resulted from those discovered polled progenies in a Sidrap ranch, were then selected and used as a breeding bull here. However, the studies in Pattalassang had to terminate due to an unexpected management constraint. All experimental animals had to be transferred to another University teaching farm in Maiwa, Enrekang District. In 2004, polled Bali cattle had been yielded here at a laboratory level. The number of polled offspring had continued to increase up to 16 heads by 2015 in the Maiwa breeding center. These types of variants had been officially declared as a selected beef cattle breed of Indonesia (Ramadhan, 2016; Syarif *et al.* 2019).

### Polled Bali cattle developed at the Hasanuddin University Breeding Centre

As previously many authors described that a variant of polled Bali cattle that had been identified earlier in a commercial beef cattle ranch in Sidrap district might be naturally hornless progenies of homozygous generations. Although the dominant inhibition of horn morphogenesis in *Bovidae* was discovered more than 70 years ago, and the causative mutation was mapped almost 20 years back, its molecular nature remained many unknown (Medugoracet *al.* 2012). Similarly, polledness traits in the recognized hornless variant of Bali cattle crosses in this study, the main causative mutation in the Bali cattle-cross breed may possibly be derived from the other party.

Nowadays, commercial large ruminant farms whether dairy or beef cattle herds are commonly confined to barns or fenced-in enclosures, such as pastures or corrals. Under these conditions horn is not only of little value, but also may lead to cause considerable economic losses due to a higher risk of injuries and any other related possible consequences i.e. infections or carcass deterioration. Therefore, in modern cattle husbandry removing horns at a very early age had previously become accepted management practices. However, all used methods are now debatable at least because of animal welfare implications (Graf and Senn, 1999). Hence, breeding polled cattle may constitute a non-invasive option to replace the common practice by means of genetic selection.

A physical feature of a male PBC resulted at the Meiwa breeding centre is presented in Figure 1. The animal showed to gain its total body weight as approximately 350 kg, when even it was only two and half years of age. Although the cattle appeared to have reached its sexual maturity, of which was indicated by its hair colour change from light-brown into a darker one, but its body weight seems to have not been fully grown yet. This is one of potential productivity advantages in Bali cattle breed that can be exploited for the development of Indonesia native beef cattle and this seems to require further studies.



Figure 1. A young male polled Bali cattle (PBC) variant when aged two and half years, Body weight approximately 350kg.

## HORNED, POLLED AND SCURRED GENETIC VARIANTS OF CATTLE

Horns are paired appendages on the head of bovine species, comprising an inner bony core and outer keratin sheath. The horn bud forms during early fetal development, but ossification of the developing horn does not occur until approximately one month after birth. However, little is known about the genetic pathways that lead to horn growth. Whereas, the polled phenotype of *Bovidae* have been considered as being controlled by a dominant inhibitor (Dove, 1935), where is characterized by the complete absence of corneous appendices (White and Ibsen, 1936). Furthermore, the naturally hornless trait or polledness in cattle is known to be controlled genetically in the chromosome. Such a polled genetic locus for cattle was firstly localised in bovine chromosome as *Bostaurus* type 1 (BTA1) by linkage mapping (Georges *et al.* 1993), and the position was later refined to the centromeric region in several studies. Four DNA sequence variants have subsequently been identified on BTA1 that are associated with the polled phenotype namely Celtic POLLED (P<sub>C</sub>), Friesian POLLED (P<sub>F</sub>), Mongolian POLLED (P<sub>M</sub>), and Guarani POLLED (P<sub>G</sub>) (Medugorac *et al.* 2012; Allais- Bonnet *et al.* 2013; Medugorac *et al.* 2017; Utsunomiya *et al.* 2019). All known variants are dominant, and cattle carrying a single POLLED variant will be either polled or scurred, depending on their genotype at the SCURS loci. It is likely that other unidentified POLLED variants exist in different populations and breeds.

The POLLED genetic locus for cattle was first localized to bovine chromosome 1 (BTA1) by linkage mapping (Georges *et al.* 1993), and the position was later refined to the centromeric region in several studies (Schmutz *et al.* 1995; Brenneman *et al.* 1996; Harlizius *et al.* 1997). Four DNA sequence variants have subsequently been identified on BTA1 that are associated with the polled phenotype: Celtic POLLED (PC), Friesian POLLED (PF), Mongolian POLLED (PM), and Guarani POLLED (PG) (Medugorac *et al.* 2012; Allais-Bonnet *et al.* 2013; Rothammer *et al.* 2014; Medugorac *et al.* 2017; Utsunomiya *et al.* 2019). All known variants are dominant, and cattle carrying a single POLLED variant will be either polled or scurred, depending on their genotype at the SCURS loci. It is likely that other unidentified POLLED variants exist in different populations and breeds. In actual fact that naturally hornless cattle or POLLED variants are inherited through an autosomal dominant mode. Whereas, permanent horns in animals including ruminants such as cattle, during ancient times appeared to be really required for them for their self-defense against predators in the wilderness. Naturally, the horns are physiologically developed from two main components comprising an outer keratin-layer and a bony pneumatized core (Dyce *et al.* 2002). Anatomically cattle horns arise from subcutaneous connective tissue (under the scalp) and later are fused to the underlying frontal bone. (Gottschalk *et al.* 1992).

Genetic background of horn growth in cattle has been studied in depth, however little is remained unknown particularly on the morphological features of bovine horn growth. Using tissue transplantation (as reviewed by Capitan *et al.* 2011), it was shown that the bony core is actually not an outgrowth of the skull, but originates from a separated centre of ossification located in the dermis and hypodermis of the calves horn bud; (ii) the keratinization of the horn bud epidermis does not induce ossification of the underlying dermis and hypodermis and conversely, thus both phenomena are probably programmed during embryogenesis; (iii) the ossifying hypodermal tissue induces the frontal bone to grow upward and to form the base of the horn spike, then it fuses with the skull by dissolving it locally (Dove, 1935). Thus, it was concluded that horn development is the result of differentiation and remodeling of various tissues originating from two distinct germ layers: ectoderm and mesoderm (Dove, 1935). Later on,

histological changes have been described in fetuses with a neck-rump length of 5.2 cm, which show a thickening of the epidermis in the region of the horn bud (Dove, 1935). During development, the horn bud region gets indented macroscopically while histologically hair follicles, sweat glands and sebaceous glands are visible. In foetuses with at neck-rump length around 61 cm the further thickening of the epidermis is accompanied by an atrophy of the adnexal structures. Nevertheless, macroscopically a whorl of hairs is visible at the time of birth (Dove, 1935).

Horns are the pairs of hard, bonelike, permanent growths projecting from the heads of cattle. The horn itself consists of dense keratin and elongates from its base. Variations in level of nutrition of the animal are reflected in variations in rapidity of horn growth, resulting in a series of rings on the horn, which may reflect seasonal stress, notably the stress of calving in cows. The age of the animal may be estimated by counting the rings of the horns.

The horn bud starts to form during the first two months of life. The horn is produced at the corium, the area of cells located at the junction of the horn and skin. If the horn but not the corium is removed, the horn will resume growing. In calves, up to about 2 months of age, the horn bud is free-floating in the skin layer above the skull. As the calf grows older, the horn bud attaches to the skull, more precisely to the periosteum of the frontal bone overlying the frontal sinus. A small horn then starts growing, then once the horn bud attaches to the skull, the horn core becomes a bony extension of the skull, and around the age of seven to eight months the hollow centre of horn core opens directly into the frontal sinuses of the skull (Parsons and Jensen, 2006). A radiography visualization of horn attachment with the skull is presented in Figure 2.



Figure 2. A radiograph visualization of bovine horn attachment with the skull: 1) the skull; 2) *frontal suture*; 3) attachment between *frontal* and horn; 4) *sinus frontal* and 5) horn (adopted from Capitan *et al.* 2011).

## HORNLESS CATTLE BREED AND INDUSTRIAL LIVESTOCK FARMING

An industrial livestock farming, where a large number of farm animals including beef and dairy cattle are reared in concise vicinity, has been growing faster than any other system. From 37 percent of world meat production in 1991-1993 had increased to 43 percent in 1996. One-tenth of the global beef productions have come from industrial production. As industrial livestock farming operations are mainly confined to barns or fenced-in enclosures such as



pastures or corrals, cattle with horns appeared to be not only of little value but also may lead to considerable economic loss due to a higher risk of injuries and the possible consequences (infection, carcass deterioration *etc.*). Therefore, in modern cattle husbandry removing horns at an early age of farm animals has become an accepted management practice (Medugorac *et al.* 2012). However, all used methods are debatable, not only because of solely animal welfare implications (Graf and Senn, 1999) but also substantial additional cost for dehorning appeared to be not economically feasible. Hence, breeding polled cattle may constitute a non-invasive option to replace the common practice by means of genetic selection.

### GENETIC SELECTION FOR BREEDING POLLED CATTLE VARIANTS

In most cattle breeds with a pair of permanent horns, there is actually a phenotypic expression of *horn/poll* gene action, where is a simple **recessive** with the polled allele (P) being dominant to the horned allele (p). Each parent has its own pair of alleles at each gene and they pass on one of these alleles for each gene to their progeny; such a progeny gets one allele from the bull and one allele from the cow to make its pair. What this means is that if a calf gets a *polledness* allele from either parent then it will be polled. If it gets two *polledness* alleles it is considered homozygous polled variant; if it gets one *polledness* and one horn allele it will be physically polled, but it will be referred to as heterozygous polled or a carrier; if it gets two horned alleles it will be homozygous horned and will be horned (See Diagram 1).

Genotype	Phenotype
PP	Polled
Pp	Polled (Scurs Possible*)
pp	Horned

\*Expect a higher probability in males than females.

Diagram 1. Possible genotypic combinations and phenotypic appearance of cattle for the *poll(P)/horn(p)* gene in cattle (Adopted from Bullock, 2019).

Like in other bovine species, naturally developed horns were historically required by the species for self-defence against predators and also to exert dominance for food and mating (Kiltie 1985; Lundrigan 1996; Davis *et al.* 2011). From the time of early domestication, both in *Bostaurus* and *Bosindicus*, the shape and size of horns have become the prominent feature of their unique phenotypic diversity among the breeds (Ajmone-Marsanet *al.* 2010). As previously highlighted, that genotypically expression as horned or hornless was controlled by *horn/poll* gene action. Generally, the head phenotype in cattle is termed as horned when a pair of recessive HORNED homozygous genes (p) are present, so that keratin coated permanent pointy protrusions anchored to the skull. Scurred when there a pair of heterozygous comprising dominant POLLED (P) and recessive HORNED (p) genes are presence, so that heterudimentary horns are loosely attached to the skin rather than the skull, such a phenotype was once detected in some progenies of inbred-Bali cattle in Brunei Darussalam (Moekti 2017, unpublished data). Whereas when called a polled variant (PP genes) if there is a complete absence of horns and scurs (Bullock, 2019). Absence and presence of horns or scurs is determined during prenatal

development. However, the postnatal development of horns versus scurs is very difficult to differentiate at an early age because both present initially as free-floating horn-buds and subsequently, only the former fuses to the cranium (Dove 1935; Wiener *et al.* 2015).

Desirability of horns has changed over the centuries with developments in the intensity and practices of both raising beef and dairy cattle (Zeder 2008; Ajmone-Marsan *et al.* 2010). In the modern commercial cattle industry, horned animals are less desirable because they pose potential hazards for other cattle and animals used for mustering (*e.g.* horses, dogs), feeding, handling and transport facilities, and/or even to farm workers. Furthermore, there is evidence that horned cattle are associated with higher costs of on-farm and post-farm production and greater risk of reduced meat and skin quality (Bunter *et al.* 2013; Knierim *et al.* 2015; Schafberg and Swalve 2015). Therefore, polled cattle have generally become much more desirable than those ones with horns.

Archaeologically, there had been an evidence suggesting that polled cattle have existed in various civilizations for at least a few centuries back (Kysely 2010; Lauwerier 2015; Schafberg and Swalve 2015). A range of different surgical, chemical and cautery methods are used to either remove unattached horn buds (disbudding) or horns attached to the cranium (dehorning). All procedures are costly and cause varying degrees of pain and reduced animal welfare, morbidity, mortality and reduced productivity, with dehorning often resulting in exposure of the frontal sinus (Knierim *et al.* 2015; Herskin and Nielsen 2018).

The underlying genes and causal mutations for horns, scurs and polledness remain to be fully elucidated. Phenotypic penetrance suggests that the poll gene is dominantly inherited, *i.e.*, PP (polled), pp (horned), with heterozygous animals usually polled but also commonly scurred (Pp). The genetic basis of scurs remains unclear although evidence suggests the condition is genetically complex and affected by polled status as well as sex of individuals (Capitan *et al.* 2009; Capitan *et al.* 2011; Tetens *et al.* 2015). In addition, an as yet unidentified African horn gene has been speculated as a possible explanation for the epistasis-like complexity in the horn inheritance in several breeds (White and Ibsen 1936; Prayaga 2007). However, no empirical evidence has been presented to confirm its existence to date (Grobler *et al.* 2018).

Genetic heterogeneity has been found across breeds linking the poll characteristics with different sequence variants of deoxyribonucleic acid (DNA), as previously mentioned namely, *Celtic* ( $P_C$ ), *Friesian* ( $P_F$ ), *Mongolian* ( $P_M$ ) and *Guarani* ( $P_G$ ) mutations (Medugorac *et al.* 2012; Utsunomiya *et al.* 2019).

These genetic mutations ( $P_C$ ,  $P_F$ ,  $P_M$  and  $P_G$ ) are believed to be not directly involved in gene coding, although putative causal effects have been reported by introgression of the  $P_C$  allele by means of gene-editing of bovine embryos that resulted in the birth of healthy and phenotypical unremarkable polled cattle (Carlson *et al.* 2016). The mutations may be involved in gene regulation and translation processes through unconventional mechanisms as speculated by the presence of antisense sequences caused by similar insertions disturbing normal function of horn growth associated genes (Allais-Bonnet *et al.* 2013). However, their association with polledness provides opportunities for genetically selecting animals to produce naturally polled cattle (Prayaga 2007; Spurlock *et al.* 2014). Notably,  $P_C$  and  $P_F$  are the most frequent mutations observed in the majority of breeds in production systems globally, and hence are the focus of this study.

## TESTING POLLED LOCUS USING MICROSATELLITE (MSAT) MAKERS OR SINGLE NUCLEOTIDE POLYMORPHISM (SNP)

Selective breeding for polled animals requires accurate early-in-life prediction of horn phenotype (Scheper *et al.* 2016). Horn phenotype is a qualitative trait controlled by genetics – 000483-9913 (OMIA 2019) –which have been mapped to bovine autosome 1 (BTA1) (Mariasegaram *et al.* 2012). To address this problem different approaches to genetic testing which provide accurate early-in-life prediction of horn phenotype have been evaluated, initially using microsatellites (MSAT) and more recently single nucleotide polymorphism (SNP).

The POLLED locus contains several microsatellite (MSAT) markers, unique to several breeds that have been successfully employed to predict the poll phenotypes (Mariasegaram *et al.* 2012). However, within-population instability and cross-population diversity of these obsolescent genetic markers make them vulnerable to diminishing accuracy over time and in non- ascertained populations. As genome sequencing technologies have become more accessible and cost effective, SNP-based testing has replaced MSAT testing. Furthermore, SNP-based indirect tests have been developed to predict both  $P_C$  and  $P_F$  alleles and are present on many commercial genotyping assays designed for genomic evaluation to reduce testing costs. However, the accuracy of commercially available SNP-based poll gene tests is constrained because their initial development only involved a limited number of taurine cattle breeds (Grobler *et al.* 2018). To address the problem that the current commonly used SNP haplotype translations are not well suited to several beef breeds, including Brahman and Brahman-infused cattle breeds, of which are common in northern parts of Australia and other tropical countries, an optimized poll gene test was developed using the recently identified SNPs associated with Celtic and Friesian mutations, and a resource herd with accurate phenotypic and genotypic recording across generations. Studies on both SNP and MSAT based testing for use in Bali cattle breed have however been appeared to be untouchable, so that further explorations are still required.

### CONCLUSION

Having been reviewed all related literatures on both genotypic and phenotypic backgrounds of polled cattle variants, there are a number important points to be taken into considerations as follows,

- 1). Even though scurred Bali cattle might be exploited as a source for POLLED gene, but the most relevant gene to control the hornless phenotype, the gene has two alleles with only the POLLED (P) one that is dominant.
- 2). The presence of polledness depends on the breed with some main beef breeds being completely polled but in most main breeds only a few numbers of heterozygous bulls are available. Many problems still need to be solved as low breeding values, presence of SCURRED alleles or negative traits.
- 3). Classical introgression programs usually take 20 years and there might have still a gap in genetic merit between horned and dehorned animals. To reduce the loss of genetic merit of polled animals and some time needed for introducing the polled gene, genomic selection, which seems to be the only promising method? Therefore, in the main breeds, it can be hypothesized that sufficient polled bulls with a high value can be obtained in approximately ten years.
- 4). The future of polled cattle is hard to predict. It will mainly depend on the acceptability by citizens, efforts made by breeding companies and availability of high breeding value bulls for farmers.



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