POLL TESTING EFFICIENCY, ACCURACY AND TRENDS IN AUSTRALIAN CATTLE

I.A.S. Randhawa[1], M.R. McGowan[1], L.R. Porto-Neto[2], B.J. Hayes[3], K.M. Schutt[4] and R.E. Lyons[1,4]

[1]School of Veterinary Science, University of Queensland, Gatton, QLD, 4343 Australia
[2]Agriculture and Food, CSIRO, St Lucia, QLD, 4067 Australia
[3]Centre for Animal Science, QAAFI, University of Queensland, St Lucia, QLD, 4072 Australia
[4]Neogen Australasia, University of Queensland Gatton campus, Gatton, QLD, 4343 Australia

SUMMARY

Poll testing is becoming common practice in Australia because it helps early prediction of head-phenotypes in calves to avoid dehorning and disbudding. It further improves cattle welfare by selecting breeding animals which are not carriers of horn alleles to avoid horned or scur calves, which may undergo physical dehorning. Current testing assays are limited to some breeds and often give inconclusive outputs as "Not Determined" or "No Results", because of ascertainment bias and marker failures. This study presents comparison of previously used poll testing assays (microsatellite and SNP based) with an optimized poll test (OPT) and poll allele distribution in beef breeds harbouring the Celtic and Friesian mutations.

INTRODUCTION

Cattle (*Bos taurus* and *Bos indicus*) species naturally evolved as horned and grow horns of different shapes and sizes as a unique phenotypic diversity between the breeds (Ajmone-Marsan *et al.* 2010). Modern cattle have further evolved their head-status as, *horn:* permanent pointy appendages attached to the skull, *scur:* pseudo horns loosely attached to the head-skin, or *poll:* complete absence of horn and scur (Wiener *et al.* 2015). Current management of horns in cattle production systems poses both welfare and economic challenges. Presence of horns poses potential hazards for other animals (injuries, damaged hides and bruised carcass), buildings, equipment and transport, and farm workers. Growth of horns can be avoided by physically dehorning. However management practices to remove horns or stop their growth remain invasive and painful for the animals (Knierim *et al.* 2015). Surgical dehorning affects growth and increases risks of infection and subsequently causes production loss and mortality, while there are also risks to workers and increased labour costs (Bunter *et al.* 2013). Genetically, the presence of horn is a qualitative trait which has been mapped on chromosome 1 (Mariasegaram *et al.* 2012). Although the genetic mechanisms underpinning *horn*, *scur* and *poll* status remain to be fully understood, inheritance of the conditions suggest that *poll* is the dominant gene, i.e., PP (polled) and pp (horned), and Pp animals generally present as poll or scurs (Capitan *et al.* 2011; Tetens *et al.* 2015).

Commercial DNA diagnostics for *poll* status are rapidly increasing and is routinely practiced by cattle farmers in Australia for informed and strategic breeding plans to reduce dehorning and disbudding. Microsatellite (MSAT) markers were used to establish the first-generation of poll testing assays (Mariasegaram *et al.* 2012). A total of 14 microsatellite markers have shown strong associations with polledness across different populations. In the poll-haplotype diagnostic test, 8 MSATs were initially used. However, the updated haplotype test contains 10 MSATs (Piper *et al.* 2014). MSAT single marker and haplotype assays were generally successful in Brahman and *Bos taurus* breeds respectively. The second generation of *poll* testing is single nucleotide polymorphism (SNP) based, and is rapidly replacing MSATs as SNP genotyping technologies become more accessible and cost

effective. SNP testing has only become available with sequencing of chromosome 1 which has identified genetic heterogeneity across breeds linking the polledness with 4 distinct insertion-deletions at the poll locus, called, Celtic (Pc), Friesian (Pf), Mongolian (Pm) and Guarani (Pg) (Medugorac *et al.* 2012; Rothammer *et al.* 2014; Wiedemar *et al.* 2014; Wiener *et al.* 2015; Utsunomiya *et al.* 2019). Notably, Pc and Pf alleles are prevalent in most of the naturally polled cattle. Predictions in SNP-based diagnostic assays rely on genetic linkage between the contiguous markers in the *poll* locus harbouring Pc and Pf (Medugorac *et al.* 2012; Rothammer *et al.* 2014; Wiedemar *et al.* 2014). Up to 10 SNPs with strong LD with the known *poll* alleles (Pc and Pf) are available in various cattle breeds and the current poll testing (CPT) assays include 5-8 SNPs.

Poll testing assays help horn management in cattle herds by early predictions of head-status. However, some breeds are disadvantaged because of ascertainment bias, marker types and other factors relating to these diagnostic tools (Connors *et al.* 2018). Here, we have investigated the efficiency and limitations of available assays which use MSATs or SNPs and propose an optimized poll testing (OPT) to efficiently diagnose the presence of Pc and Pf across 10 breeds of cattle.

MATERIALS AND METHODS

Animal ethics approval for tail-hair samples, head phenotypes, genotyping and sequencing were obtained (AEC # SVS/301/18). Genomic data of 37,694 animals across ten breeds (Table 1) was used to compare the available poll test results using MSATs (n=20,534) and SNPs (n=18,793) based assays, and with the proposed SNPs-based OPT (n=18,793). To assess the phenotypic concordance, information about their head-status (*horn*, *scur*, and *poll*) from 6,930 (out of 18,793) registered animals of 8 breeds in Australia (excluding Angus and Wagyu) were obtained from the BREEDPLAN database (http://breedplan.une.edu.au/index.php). Hair samples of Brahman (n=60 out of 2691) were used from available stocks for targeted DNA sequencing. In addition, collection of hair samples and assessing of head-status of Droughtmaster (n=84) cattle from UQ's research herd were performed for phenotypic and genetic concordance for validation.

First, we compared the efficiency of available MSAT and CPT assay based predictions using available poll test results on different samples, because most animals were tested with either MSATs or SNPs based markers. Second, 10 SNPs in the poll region were investigated for genotyping failures, monomorphism and overall informativeness to develop the optimized poll testing (OPT). Third, OPT based predictions were evaluated for phenotypic concordance with BREEDPLAN data (available for 6,930 registered animals only) and finally, validated by UQ-herd.

Table 1. Breed samples tested with microsatellite (MSAT) and SNP-based assays

Breeds	Samples	MSAT tested	SNP tested	Tested by both
Angus	1630	28	1602	0
Brahman	7009	4532	2691	214
Brangus	754	745	37	28
Charolais	3148	2666	900	418
Droughtmaster	2611	2223	708	220
Hereford	6424	3485	3341	402
Limousin	2193	2124	207	138
Santa Gertrudis	4427	4306	136	15
Shorthorn	316	224	121	29
Wagyu	9182	201	9050	69
Total	37,694	20,534	18,793	1,533

RESULTS AND DISCUSSIONS

We found that MSAT-based testing failed to predict genotypes (HH, PH or PP) for *horn* or *poll* conditions in 11.7% of tests performed across all breeds combined and these were reported as Not Determined (Figure 1). The CPT diagnostic test significantly reduced the frequency of "No Results" being reported, with the notable exception of Zebu (*Bos indicus*) and their cross-bred cattle which were still constrained by No Result issues (Figure 1). Out of 18,793 SNP-tested samples, 5.48% had failed to identify an unambiguous genotype with CPT and hence were predicted as No Result with the majority of these no results being Brahman (18.3%) and Brangus (22.2%).

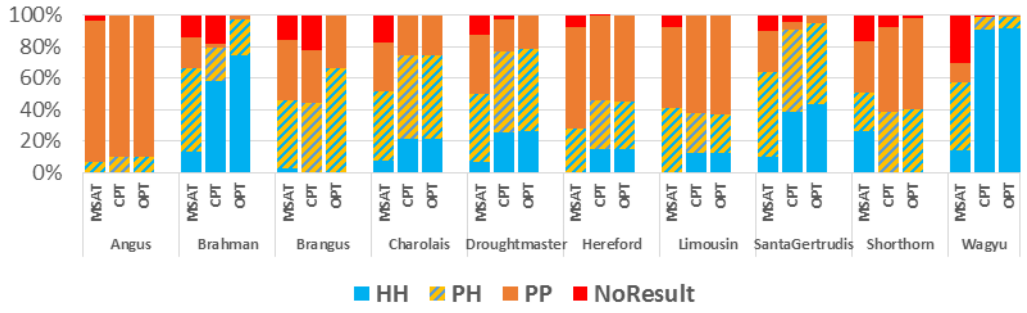

Figure 1. Comparison of poll testing by microsatellites (MSAT) and SNP-based current poll test (CPT) and optimized poll test (OPT) in 10 breeds of Australia

Utility of 10 SNPs was investigated for prediction of Pc and Pf allele prevalence in different breeds. Initially, a single SNP (rs800947704) was found to be failing in genotyping assays in 8 of the 10 breeds (n=662 out of 16,828), especially in Brahman which had a 14.4% failure rate. Targeted sequencing of a 1,098 bp (1,654,527-1,655,625) fragment surrounding rs800947704 in Brahman samples showed that the probe region (within 50bp of target SNP) was unstable causing genotyping failure. Hence the SNP was rejected as a useful marker. Further investigation found that 4 other SNPs were not reliable for accurate predictions, of which 2 SNPs (rs798116945 and rs800767839) were highly monomorphic and 2 SNPs (rs799187101 and rs799920960) were not in complete LD. The No Results predictions were caused by issues with 1 or more of these 5 SNPs, indicating that they were unsuitable for the poll testing assays in Zebu cattle. The other 5 SNPs passed the inclusion criteria for the OPT predictions and were evaluated using 18,589 samples of European, Zebu and their cross-bred populations. Previously successful predictions (n=18,019) were found to remain unchanged (100%) using OPT relative to the original prediction. Of the previously unsuccessful (No Results, n=570) samples, 569 (99.8%) were effectively classified into one of the head-status predictions. Overall allele frequencies were found as H = 0.57, Pc = 0.40 and Pf = 0.03 (Figure 2). Genotype distributions (HH = 40.4, HP = 32.3, PP = 27.3) were different than phenotypic rates (*Horn* = 42.7, *Scur* = 6.2, *Poll* = 51.1), predominantly because many heterozygous animals (HP) are *poll* (Table 2). Numbers of OPT-based genotypes (and associated phenotypes as a %) in the UQ herd were; HH =15 (*horn* 100%), HP = 45 (*scur* 51% and *poll* 49%) and PP = 24 (*poll* 100%).

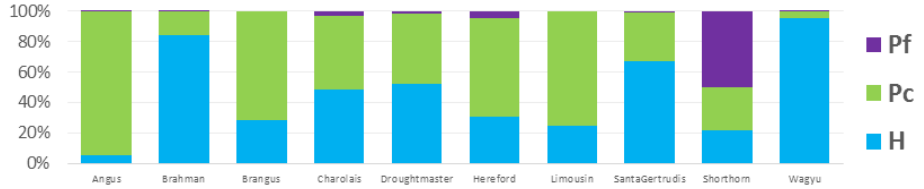

Figure 2. Frequency (%) of *horn* (H) and *poll* (Pc & Pf) alleles in 10 breeds (n=18,793)

Table 2. Concordance between OPT genotypes and BREEDPLAN phenotypes in 8 breeds

OPT genotypes	Number tested	Phenotypic concordance (%) with head-status		
		Horn	*Scur*	*Poll*
HH	2800	94.8 %	3.10 %	2.10 %
HPc	2121	13.5 %	15.7 %	70.8 %
HPf	120	2.50 %	5.80 %	91.7 %
PcPc	1595	0.75 %	0.25 %	99.0 %
PcPf	267	0.37 %	0.37 %	99.2 %
PfPf	27	-	-	100 %

OPT-based genotypes have shown high concordance with known head-status, except for HPc and HPf that can result in *scur* phenotypes, with indications in literature pointing to the probability that sex (female) and sex hormones (steer) sway heterozygotes to be *poll* (Randhawa *et al.* 2019). It is very unlikely that HH animals can be either *scur* (3.1%) or *poll* (2.1%). However, inaccuracies with phenotypic recording are common (Connors *et al.* 2018). Overall, using the OPT can effectively resolve MSAT and CPT limitations to accurately predict true *poll* conditions (over 99%) in *Bos taurus*, *Bos indicus* and cross-bred beef cattle. We continue to investigate the genetics of the *scur*.

ACKNOWLEDGEMENTS

This study is funded by the Meat and Livestock Australia, project L.GEN.1713.

REFERENCES

Ajmone-Marsan P., Garcia J.F. and Lenstra J.A. (2010) *Evol Anthropol* **19**: 148.

Bunter K.L., Johnston D.J., Wolcott M.L. and Fordyce G. (2013) *Anim. Prod. Sci.* **54**:.25.

Capitan A., Grohs C., Weiss B., Rossignol M.-N., Reversé P. and Eggen A. (2011) *PLoS One* **6**:.e22242.

Connors N.K., Tier B. and Johnston D.J. (2018) Current status of Australia's diagnostic poll haplotype test. *Wld Congr. Genet. Appl..Livest. Prod*. 1**1:** 344.

Knierim U., Irrgang N. and Roth B.A. (2015) *Livestock Sci.* **179**:.29.

Mariasegaram M., Harrison B.E., Bolton J.A., Tier B., Henshall J.M., Barendse W. and Prayaga K.C. (2012) *Anim. Genet.* **43**:.683.

Medugorac I., Seichter D., Graf A., Russ I., Blum H., Göpel K.H., Rothammer S., Förster M. and Krebs S. (2012) *PLoS One* **7**:.e39477.

Piper E.K., Tier B. and Henshall J.M. (2014) *Wld Congr. Genet. Appl. Livest. Prod.* **10**: 1.

Randhawa I.A.S., Lyons R.E., Hayes B.J., Porto-Neto L.R. and McGowan M.R. (2019) *Proc. Assoc. Advmt. Anim. Breed. Genet.* **23**: .

Rothammer S., Capitan A., Mullaart E., Seichter D., Russ I. and Medugorac I. (2014) *Genet. Sel. Evol.* **46**: 44.

Tetens J., Wiedemar N., Menoud A., Thaller G. and Drögemüller C. (2015) *Anim. Genet.* **46**: 224.

Utsunomiya Y.T., Torrecilha R.B.P., Milanesi M., Paulan S.d.C., Utsunomiya A.T.H. and Garcia J.F. (2019) *Anim. Genet.* **50**: 187.

Wiedemar N., Tetens J., Jagannathan V., Menoud A., Neuenschwander S., Bruggmann R., Thaller G. and Drögemüller C. (2014) *PLoS One* **9**: e93435.

Wiener D.J., Wiedemar N., Welle M.M. and Drögemüller C. (2015) *PLoS One* **10**: e0127691.