HIGH ALTITUDE DISEASE AND GENETICS OF BEEF CATTLE AT HIGH ELEVATION REGIONS

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1 Introduction

In high altitude states such as Colorado, Wyoming, New Mexico, and Utah, bovine pulmonary hypertension (BPH) is observed and commonly referred to as "brisket disease" or "high altitude disease (HAD)" (Holt and Callen, 2007). The disease was first studied by Glover and Newsome (1915) in cattle for the sole purpose of advising Colorado and New Mexico stockman to protect their herds. The cardinal sign of HAD is swelling of the brisket due to fluid accumulation in the thoracic cavity. It is believed that in response to alveolar hypoxia, the pulmonary artery constricts resulting in hypertension, right heart ventricular hypertrophy, vascular remodeling, and death from congestive heart failure (Holt and Callan, 2007). Pulmonary arterial pressure (PAP) is a measure indicative of hypertension and has been reported to be moderately heritable in cattle (0.34 to 0.46; Enns et al., 1992; Shirley et al., 2007). Therefore, PAP has been widely used as an indicator trait of BPH/HAD in recent studies. Therefore, various studies involving PAP have been applied to describe reasons for HAD, including genetics, since 1914.

2 Literature review

2.1 Economics

Why HAD is important and worth to be studied in cattle at high elevation? There is a high economic relevance to HAD, with an incidence of 3% to 5% typically in native cattle (Holt and Callen, 2007). However, it is a major cause of calf morbidity for beef cattle ranches and feedyards above 1500 m (Hecht et al., 1962; Jensen et al., 1976). A producer losing 20% of his 600 calves equates to $78,864 of lost potential income between summer turnout and weaning based on the market price ($1.24/lb. live weight, November 7th 2011) and the herd average weaning weight in 2009 (529.8± 72.4lbs; Neary, 2013). Native cattle at high altitude may be more resistant to HAD than low attitude cattle due to artificial selection (Will et al., 1975). About 10% to 40% of cattle develop HAD when they were moved from low altitude to high altitude (Grover et al., 1963, Will et al., 1970).

2.2 Physiology of HAD

Based on clinical and physiologic principles, three major high-altitude diseases were identified (West, 2004): 1. Acute mountain sickness. The mechanisms are not fully understood, but brain swelling may be a phenotype. 2. High-altitude pulmonary edema. The mechanism is probably uneven hypoxic pulmonary vasoconstriction that exposes some capillaries to a high pressure, damaging their walls and leading to a high-permeability form of edema. 3. High-altitude cerebral edema. It closely related to acute mountain sickness and that it is the extreme end of the spectrum. These are specific description of different types of High Altitude Disease. What can be used as a general reference to identify HAD? The hypoxia from the high elevation regions is the major cause of HAD/BHP. Alexander and Jenson (1959, 1963) found that, hypoxia at high elevation causes pulmonary vasoconstriction, increased pulmonary arterial pressure (PAP), right ventricle stress, congestive right heart failure, and hydrothorax in the chest cavity and brisket. Additionally, Holt and Callen (2007) indicated that HAD is characterized by the presence of ventral edema in the brisket region secondary to increased vascular hydrostatic pressure (intravascular hypertension) and the loss of fluid into the extra vascular space.

2.3 Relationship between PAP and HAD

As an indicator of HAD, PAP scores were used to

assist selection of cattle to reduce HAD in recent decades in high altitude regions. Holt and Callen (2007) indicate that: 1. The measured PAP of less than 41 mmHg at an elevation greater than 1500 m (5000 ft) are likely to maintain an acceptable PAP at high altitude and serve as good breeding stock; 2. Animals with measured PAP larger than 41 mmHg and less than 49mmHg at high altitude should be used with caution at high elevations; 3. Cattle with PAP larger than 49mmHg at any altitude are at risk for developing HAD and should not be maintained or used in breeding programs at high altitude. Therefore, these recommendations serve as phenotypic selection tools. Also, the information indicates that higher PAP measures imply higher risk for HAD.

2.4 Measurement of PAP

The procedure used to measure PAP has been used for more than 30 years. However, PAP measures can be influenced by any unprofessional action in the process. Therefore, the PAP score can only be taken by one licensed veterinarian in one herd in order for selection to be more effective. With the right equipment and facilities, a professional veterinarian can take PAP score for a large number of animals daily, which makes PAP a measurable and affordable trait for selection. The PAP test is a right heart catheterization procedure, which requires jugular venipuncture, catheter insertion and passing flexible catheter tubing through a large bore needle inserted into the jugular vein. The catheter is passed down the jugular vein, through the right atrium, into the right ventricle, and then into the pulmonary artery. Once the catheter is inside the pulmonary artery, an average blood pressure (average of systolic and diastolic values) is recorded from the heart monitor, which is attached to the catheter via a transducer (Ahola et al., 2007).

2.5 Genetic Parameters for PAP

2.5.1 Heritability and Repeatability

In order to reveal the genetics influences within HAD, heritability, repeatability and genetic correlation related to PAP have been estimated in many studies. Heritability is the proportion of phenotypic variation that is explained by additive genetic variation. Table 1 summarized the heritability of PAP reported in previous literature. Pulmonary arterial pressure (PAP) has been shown to be moderately to highly heritable and repeatable in cattle (Schimmel, 1981; Enns et al., 1992; Shirley et al., 2007). The heritability and repeatability of PAP were first estimated in a dissertation work of Schimmel (1981). The PAP values in this study were collected from weaning calves and mature cow raised at the San Juan Basin Research Center, Hesperus, Colorado (elevation at 2,316m). He reported heritabilities of PAP as 0.77 ± 0.21, 0.60 ± 0.24, 0.40 ± 0.13 and 0.13 to 0.23 for bull, heifer, calves and cows. Enns (1992) reported a heritability estimate as 0.46 ± 0.16 of weaning measured PAP, which were from Angus cattle from western Colorado. The most recent published heritability of PAP was 0.34 ± 0.05 reported by Shirley (2007). In addition, an ongoing study estimated heritability for PAP measured in yearling Angus cattle was 0.21 ± 0.04, 0.37 ± 0.08, 0.19 ± 0.14 and 0.23 ± 0.03 for bulls, heifers, steers and compiled data (Cockrum, unpublished data). Similar to many other traits, the estimated heritability was varied among studies, which may account for the genetic by environmental effect of age of PAP and sex management. However, all of the studies showed a moderate to high heritability for PAP measure (0.23 to 0.77). Furthermore, the study from Cockrum, which will be published in August 2014 at 10th World Congress on Genetics Applied to Livestock production (WCGALP), was similar to results from Schimmel (1981), in that the heritability of PAP of bulls was higher than that of heifers. This fact may result from the high intensity artificial selection of bulls. The repeatability reports were limited in previous studies, the reason for which may be that the PAP score is usually measured once (i.e. yearling). However we can expect a moderate repeatability of PAP, based on the repeatability as 0.25 to 0.16 on cows reported by Schimmel (1981).

2.5.2 Genetic Correlation

Veit and Farrell (1978) suggested that larger body size and metabolic demands would place stress on the bovine pulmonary system; thus pre-disposing cattle to respiratory disease and pulmonary hypertension. This viewpoint was supported by the estimated genetic correlation from Shirley et al. (2007), who reported the genetic correlation between PAP and birth weight (BW) or weaning weight (WW) to be moderate (i.e. 0.49 to 0.51). However, the genetic correlation between PAP and post weaning growth traits of yearling weigh (YW) and post weaning gain (PWG) were reported to be 0.22

± 0.04 and 0.04 ± 0.12, respectively, which is interpreted as weak, yet positive genetic correlation (Zeng et al., unpublished data). However, Schimmel (1981) reported a genetic correlation of between bull PAP and YW. Based on these results, the genetic parameters of PAP appeared to be varying among studies.

The varied estimates of genetic correlations may be explained by genetic difference observed among PAP collected at different age (weaning versus yearling) or different sex (bulls versus heifers; Cockrum et al., unpublished data). The genetic correlation between PAP of weaning and yearling (0.56 ± 0.24), or between yearling PAP of bulls and heifers (0.67 ± 0.15) was not high, which suggested that PAP measurements at weaning and yearling, or in heifers and bulls were potentially different traits (Cockrum et al., unpublished data). These results implied a genetic difference between BPH/HAD of bulls and heifers. However, we must consider these results as potentially confound with growth management. Both of these trait measures (i.e. bulls and heifers) were based on the data from John E. Rouse Ranch of Colorado State University Beef Improvement Center (CSU-BIC). In the production system, bulls were developed within a grain-supplemented performance test, whereas heifers and steers were grazed. Therefore, these may be a genetic by environmental interaction via two source of information: 1) sex; 2) diet environment. Similarly, the environmental effect on phenotype of PAP had been reported in earlier literatures, which suggested that age, gender, temperature and diet influenced PAP phenotype (Rhodes, 2005; Holt and Callen., 2007).

2.5.3 Model Used for Genetic Evaluation

Both univariate and multivariate animal models were used in previous PAP studies. The fixed effects included in the models included PAP date, sex, age of dam, management contemporary group, and age of PAP as covariate (Shirley et al., 2007). The random effects in these models were animals. In previous studies, the major software used to execute these mixed animal models with continuous response variable was ASReml (Gilmour, 2009). However, PAP scores are not normally distributed which violate our assumption in evaluation of these models. The problem may be solved by transforming the PAP data to categorical data, and then execute a threshold animal model for genetic evaluation. We hypothesize the later is reasonable as we are most interested in the extreme value of PAP.

2.5.4 Expected Progeny Differences (EPD) for PAP

The expected progeny differences (EPD) for PAP were first estimated with data from the Tybar Ranch, Carbondale, CO. Since the first use of a PAP EPD for selection of resistance to HAD at the Tybar Ranch in 1992, the EPD for PAP was continuously used in cattle breeding in Colorado (Enns, 2011). Also, the PAP EPD has been used in the selection program in John E. Rouse Ranch of Colorado State University Beef Improvement Center (CSU-BIC) since 2006. Figure 1 presents the genetic trend of PAP EPD from both Tybar Ranch and CSU-BIC. The genetic trend in PAP score has been consistently downward (favorable) since the use of a PAP EPD in Tybar Ranch beginning in 1992. The downward (favorable) genetic trend has also been seen at CSU-BIC since the use of a PAP EPD in 2006. Producer reports collected in veterinary health studies suggest that, in some cases, low PAP cows should significantly reduce the incidence of HAD within their calf crop. However, report from other producers indicated that the selection on low PAP has no influence on reducing the mortality of pre-weaned beef calves (Neary, 2013). Therefore, more studies should be executed to ensure that genetic selection on low PAP would reduce the chance of cattle to HAD/BPH.

Although, PAP has been widely recognized as an indicator to study HAD/BPH, there are limitations in this trait and its interpretation. First, cattle need adaption period for at least 30 days before PAP scored measurement when they move from low altitude to high altitude area (Holt and Callen, 2007). Second, the measure of PAP needs to be completed by a skilled veterinarian.

2.6 Genomic wide Association Study (GWAS)

With the advance of molecular genetics techniques, high-density marker maps and tools are available and large number of animals can be genotyped with a reasonable investment. This fact allows genome wide association study (GWAS), which utilizes high-density single-nucleotide polymorphisms (SNP). The GWAS is an approach to revel common genetic variants in different individuals to assess if any variant is associated with a trait. In the beef industry, GWAS can be used in genomic selection using estimate genomic estimate

breeding value (GEBV), whose accuracy is much higher than traditional EBV. Also, GWAS has been widely used in identifying significant SNP, biological pathways and networks underlying complex traits. Therefore it is beneficial to conduct GWAS on PAP and use GEBV or marker assisted selection (MAS) to conduct selection of cattle at both low and high altitude for resistant to HAD. However, there are few published GWAS studies on PAP or HAD on cattle, except for the work from Newman et al. (2011) and unpublished work from Colorado State University to be presented in August 2014 at 10th World Congress on Genetics Applied to Livestock production (WCGALP), Vancouver, BC, Canada

2.6.1 Response Variable in GWAS

Information resources used in GWAS can be alternative sources of information including single or repeated measures of individual phenotypic performance, information on progeny, estimated breeding value (EBV) from genetic evaluations, or a pooled mixture of more than one of these information sources (Garrick et al., 2009). The SNP/marker effects were come from the training data and would be used to fit the test data to estimate the GEBV. To guarantee the accuracy of GEBV prediction, the ideal data for training would be true genetic merit data observed on unrelated animals in the absence of selection (Garrick et al., 2009). Also, as indicated previously, the PAP scores are not normally distributed, which violate the assumption of statistical methods used in GWAS. Therefore, a deregressed estimated breeding value (DEBV) may be the best response variable used in future GWAS on PAP.

2.6.2 Method Used in GWAS

Even though, published GWAS of PAP or HAD on cattle are forthcoming, the statistical methods used in GWAS for different traits are generally the same. In order to improve the accuracy of GWAS, many statistical methods have been applied during the past 20 years. Actually, these methods are different kinds of model selection methods. The most widely used methods include BLUP, BayesA, BayesB, BayeCϖ, Bayesian LASSO, GBLUP, machine learning etc. Hayes and Goddard (2010) concluded that the highest accuracies of GWAS were achieved when the prior distribution of SNP effects matches the true distribution. The method assuming many SNP effects of zero and a small proportion of SNPs with moderate to large effects yield higher accuracy GEBV.

The genome based BLUP, BayesA and BayesB were first introduced, compared and discussed in the paper of Meuwissen et al. (2001). The BLUP method assumed a normal distribution of SNP effects, which suggested a very large number of QTL with small effects. The BayesA assumed at distribution of SNP effects, which is based on a large number of QTL with small effects and a small proportion with moderate to large effects. In BayesA, the variance of each SNP effect was assumed unequal and under an inverted chi-square distribution with scale parameter S and v degree of freedom, whereas it is assumed that the error variance was under a inverted chi-square with scale parameters 2. BayesB is a method assuming mixture distribution of zero effects and t distribution of effects for SNP, which suggest a large number of genome regions with zero effect and a small proportion of QTL with moderate effects. The variance distribution assumption for QTL loci and error term are the same as BayesA.

Habier et al. (2011) developed BayesC and BayesCϖ. Both assumed that there is ϖproportion of loci have 0 effect and (1-ϖ) proportion of loci have moderate to large effect with common variance across these loci. The ϖ is a fixed value in BayesC while in BayesCϖ, ϖ is sampled from a beta distribution based on data. The error variance is assumed under an inverted chi-square distribution with scale parameter 2 as other Bayes methods. These Bayesian methods can be executed using the GenSel software (Fernando and Garrick, 2008).

Another method is Bayesian Lasso introduced by Yi and Xu (2008), which also assumed a very large proportion of SNP effect close to zero and small proportion with a moderate to large effect. In this method, the SNP effect is under a normal distribution and the variance of QTL is under an exponential distribution.

The GBLUP is based on the restricted maximum likelihood (REML) concept. The SNP effects and variance can be estimated from mixed model developed by Henderson (1976) based on REML with treating the SNP as random effect and including a genomic relationship matrix. Using this methods, fixed effects can be estimated too. This GWAS method can be accomplished using many software packages including SVS (Golden Helix, Inc., Bozeman, MT), R, SAS (SAS In-

stitute, Cary NC), ASReml (Gilmour et al., 2009), etc. In R, some GWAS packages written by other researchers can be used directly.

In addition to the previous methods, a machine learning method was developed by Long et al. (2007). This method can be used to classify suspect and healthy animals with high accuracy and identify disease related SNPs. Specifically, a case-control experiment is designed, then machine learning method was used to select SNPs. Besides the naïve Bayes used in Long's study, the machine learning method has many algorithms including support vector machine, decision tree, artificial neural machine, etc.

In recent years, a method named as multiple locus mixed model (MLMM) were used in GWAS studies. It is a method using a simple stepwise mixed-model regression with forward inclusion and backward elimination of genotypic markers as fixed effect covariates with a genomic relationship matrix (Segura, 2012). The variance components are re-estimated between each forward and backward step. Currently, the MLMM is available in the SVS (Golden Helix, Inc., Bozeman, MT).

2.6.3 Results of GWAS

Few GWAS studies have been conducted for HAD or PAP on cattle, but there are some genomic related studies on HAD for yaks and humans. Reviews of these effects give us some genomic information on HAD across species. Also, these results can be compared to our future findings to help us explain our results. The following is a review of potential candidate genes.

In the study of Simonson et al. (2010), they reported that gene Egl nine homolog 1 (EGLN1) and Peroxisome proliferator-activated receptor alpha (PPARA) were associated with hypoxia response factor (HIF) and expressed in high altitude adapted individuals, which can be used to study the high altitude adaption pathway in humans. Newman et al. (2011) provided the first molecular interrogation on BPH based on a case control GWAS and gene expression study. The study revealed six or more significant genes, among which three genes were candidates possibly involved in BPH including NADH dehydrogenase (ubiquinone) flavoprotein 2 (NDUFV), myosin heavy chain 15 (MYH15) and the myocardial signaling protein (FKBP1A). Besides identification of significant genes, BPH related pathway results and gene networks were also explained in the study to help the understanding of the biological signature of BPH. Qiu et al. (2012) found that gene families, which were related to sensory perception and energy metabolism, as well as an enrichment of protein domains involved in sensing the extracellular environment and hypoxic, were express different between yak and cattle. This fact can be used to study the adaption to high altitude in other animal species and humans. In addition, a study (Wang et al., 2012) identified a Hypoxia-inducible factor-2alpha (HIF-2□) encoding gene, Endothelial PAS domain-containing protein 1 (EPAS-1), which is a key gene mutated in the Tibetan population adapted to living at high altitude.

In future, since cattle are considered a natural animal model to study HAD and higher density chip are available for genotyping, GWAS should be done on cattle. Since the heritable PAP score was widely treated as an indicator of HAD. The GWAS study based on PAP score can be used to identify the most significant SNP related to HAD, and then related genes can be studied. The method discussed by Fortes et al. (2011) can be used to develop a gene network on PAP with the detected SNPs. Furthermore, these genes can be used to conduct a pathway study, which can help reveal the whole picture of HAD and provide efficient treatment plan.

3 Conclusion and Implications to Genetic Improvement of Beef Cattle

Selection for resistance to HAD/BPH is important for beef cattle, because HAD influences calf mortality at high altitudes (above 1500m). Pulmonary arterial pressure can be treated as indicator trait for selection of tolerance to high altitude, especially since it is physiologically related to HAD/BPH and moderately heritable. Genetic selection for low PAP by beef producers at high altitudes could potentially improve profitability by reducing the mortality rate. However, more genetic evidence is needed to ensure that selection for low PAP could reduce the incidence of HAD. The GWAS of PAP score can be used to identify the most significant SNPs or genes potentially related to HAD, and estimate GEBV to serve as a selection tool. Thus, genomic information can help the selection of cattle for resistance to HAD at earlier ages. Besides the benefit of traditional

Table 1. Estimated heritability and repeatability for pulmonary arterial pressure (PAP) in previous literature

Author	Heritability	Repeatability	Age of cattle
Schimmel (1981)	0.13~0.23	0.25~0.26	Mature Cow
Schimmel (1983)	0.20~0.77	-	Weaning
Enns (1992)	0.46(0.16)	-	166d-662d
Shirley (2007)	0.34(0.05)	-	Weaning

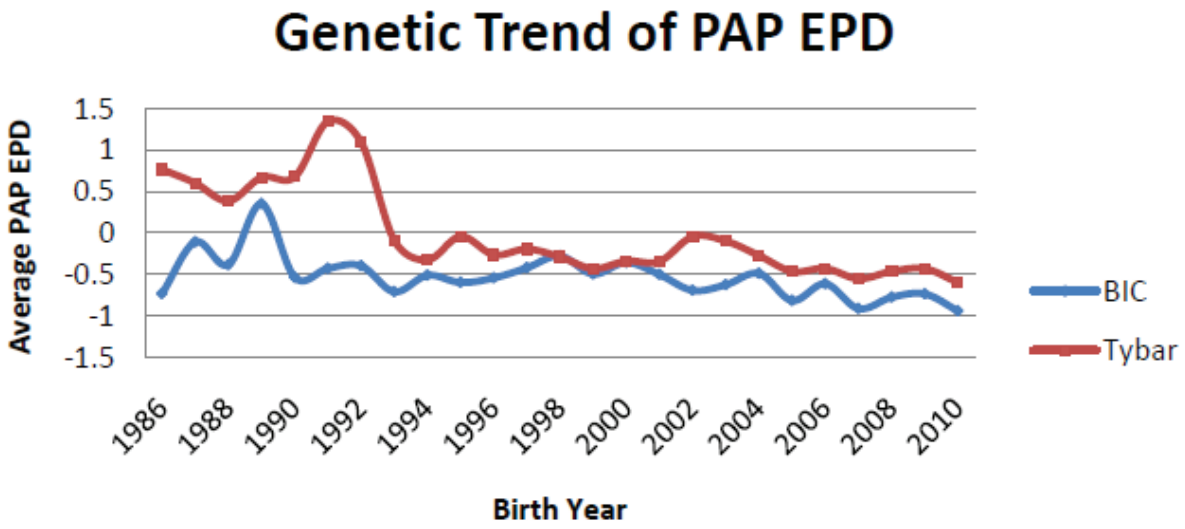

Figure 1. Genetic trend pulmonary artery pressure at the Tybar Ranch (Tybar) and the CSU John E. Rouse Beef Improvement Center (BIC) since selection with EPD began in 1992 in Tybar) and 2002 in BIC (Enns et al., 2011).

genetic selection on PAP, GWAS of PAP will also help reveal the genomic architecture of HAD by studying genes, and increase the selection efficiency for resistance to HAD. However, case/control data of HAD are needed to help expose information of the complex high altitude disease. Thus, it is important and beneficial to collaborate with breeders across mountains regions of the country to collect the HAD case in the future.

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