BOVINE LEUKOCYTE ADHESION DEFICIENCY SYNDROME (BLAD): A RECESSIVE DISORDER IN HOLSTEIN FRIESIAN CATTLE- A REVIEW

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ABSTRACT

The present review is to summarize findings on one of the most important recessive hereditary disorder Bovine Leukocyte Adhesion Deficiency Syndrome (BLAD) in cattle. It is a disease of young Holstein calves characterized by recurrent pneumonia, ulcerative and granulomatous stomatitis, periodontitis, delayed wound healing and persistent neutrophilia. The recessive homozygous form is lethal and since carrier animals have viability, BLAD frequency increases by use of carrier bulls in Artificial insemination (AI). The gene CD18 was the suspected culprit and a point mutation was eventually identified within the alleles encoding bovine CD18 in a Holstein calf affected with BLAD. This mutation causes aspartic acid to glycine substitution at the amino acid level. PCR-RFLP, PCR-SSCP, AS-PCR and Tetra-primer ARMS-PCR are certain PCR based techniques which could be used to screen BLAD in cattle. The use of these molecular technologies promises quick detection of carriers enables their culling therefore, controlling and preventing the spread of BLAD in the population.

Key words: Bovine leukocyte, Adhesion deficiency, Recessive disorder.

Bovine leukocyte adhesion deficiency syndrome is an autosomal recessive hereditary disease affecting young Holstein calves characterized by recurrent bacterial infections, progressive periodontitis, ulcers of oral mucosa, and impaired inflammatory responses (Kehrli et al., 1990; Nagahata et al., 1987). These clinical findings are associated with impaired Neutrophil functions such as markedly decreased adherence, chemotaxis, and phagocytosis (Nagahata et al., 1987; Takahashi et al., 1987). In 1990, a lack of $\beta_2$ integrin molecules expressed on the leukocytes from affected animals was found in a calf with granulocytopenia syndrome (Kehrli et al., 1990), and this disease was termed bovine leukocyte adhesion deficiency, which was considered to be analogous to human LAD. (Anderson et al., 1986; Kishimoto et al., 1989; Springer et al., 1984). Same deficiency syndrome is also called CLAD in dogs (Giger et al., 1987). BLAD causes immunodepression in the early days of life. The animal is then sensitive to all infectious agents which can cause digestive, respiratory, cutaneous disorders and death. This disease is due to the absence of a protein $\beta_2$ integrin at the surface of neutrophiles, which stops them from playing their role in non-specific defense of the animal (Gourreau et al., 1998).

History

BLAD was first identified in Holstein-Friesian cattle at the beginning of the eighties and no study has reported the occurrence and etioloogy of this disease in other breeds. The mutation found in the Holstein breed can be
traced back to a heterozygote bull (Oosbornsdale Ivanhoe), which due to its elevated genetic merit for milk production has been widely used in artificial insemination. This bull and its offspring (Penstate Ivanhoe star - son and Carlin M Ivanhoe Bell - grandson) founded one of the main Holstein lineages. They are also responsible for spreading BLAD to several herd worldwide (Shuster et al., 1992). Affected cattle with BLAD were linked to common ancestral sires that had been documented to be carriers. Several Holstein Friesian bulls were identified as BLAD carriers, and the gene encoding impaired CD18 spread to many countries.

**Molecular basis of BLAD**

The molecular basis of BLAD is a single point mutation (adenine to guanine) at position 383 of the CD18 gene (Fig 1), which caused an aspartic acid to glycine substitution at amino acid 128 (D128G) in the glycoprotein. This mutation occurs near the center of 26 consecutive amino acids that are identical in normal bovine, human, and murine CD18 and lies within a large extracellular region that is highly conserved across integrin β subunits (Kishimoto et al., 1989; Shuster et al., 1992). The other mutation, cytosine to thymine, between the normal and the BLAD allele detected at position 775 in the CD18 gene is silent (Shuster et al., 1992).

**Mutations**

As per the definition, mutation is an alteration in the DNA (or RNA) that comprises the genetic information. In case of BLAD the mutation is missense and there is an alteration in a single codon so amino acid in protein is replaced with different amino acid, but in nonsense mutation change is happening in codon from an amino acid to a stop codon (Snustad and Simmons, 2006).

**Leukocyte Integrins**

The Integrin β2 (Fig. 2) include LFA-1, CR-3 and p150, 95 (CD11C) which consist of unique subunits CD11a (180 KDa), CD11b (170 KDa) and CD11c (150 KDa) respectively and a common β subunit CD18 (95 KDa) (Anderson and Springer, 1987; Hynes, 1987; Larson and Springer, 1990). LFA-1 is expressed on all leukocytes. CR3 and p150, 95 are found on the surfaces of neutrophils, monocytes/macrophages and natural killer cells (Anderson and Springer, 1987; Springer, 1990; Kishimoto et al., 1989). These are transmembrane proteins with relatively small cytoplasmic domains. BLAD is homozygous for the D128G allele of the CD18 gene and as a result there is impaired expression of the integrin β2 (CD11a,b,c/CD18) of leukocyte adhesion molecules (Shuster et al., 1992). Specific binding of CR3 and LFA1 on the neutrophil surface with intercellular adhesion molecule 1 (ICAM-1) expressed on vascular endothelium is required for neutrophil emigration into vascular sites of inflammation (Kishimoto et al., 1989; Anderson and Springer, 1987; Nagahata et al., 1995). So integrin β2 has a direct role in immune system.

**BLAD and its relationship with other traits**

Steinholt et al. (1994) reported that autosomes from normal bulls had larger mean autosomal areas than bulls heterozygous for the bovine leukocyte adhesion deficiency (BLAD) gene (473 vs. 419+or-18 pixels). BLAD carrier bulls had spermatozoa with greater head area than normal bulls.
Lymphocyte chromosomes of individuals homozygous for BLAD were significantly smaller than those of BLAD heterozygotes.

Histological examination of necrotic lesions of the digestive and respiratory systems of HF animals revealed a lack of extravascular polymorphonuclear granulocytes despite vascular leukocytosis, although polymorphs were abundant in catarrhal bronchopneumonia lesions (Garderen et al., 1994). An experiment carried out to find out effects of the BLAD allele on milk, fat and protein yields, productive life and somatic cell scores. Data gathered from the Holstein Association USA showed that carrier bulls were negative for all traits but significant only for protein (Powell et al., 1996). Same experiment also carried out in Taiwan and found milk production of BLAD-carriers was slightly higher than normal cows but somatic cell count was worse (Huang et al., 2001).

In another investigation in Hungary the relationship between bovine leukocyte adhesion deficiency (BLAD) genotype of bulls, their breeding value for milk yield, milk fat yield and milk protein yield and the milk yield of their daughters was studied. BLAD-carrier and healthy bulls were compared on the basis of their breeding value. The first 100 bulls ranked according to the total production index were
used, including 9 BLAD carriers and 77 healthy sires with 2835 and 21950 daughters respectively. The BLAD genotype was not indicated for 14 bulls. The healthy animals significantly outperformed the BLAD carriers (Janosa et al., 1999).

**Prevalence and Frequency of BLAD**

BLAD carriers were among the most prominent bulls of the Holstein breed such as Osborndale Ivanhoe, Penstate Ivanhoe Star and Carlin-M Ivanhoe Bell (Powell et al., 1996; Shuster et al., 1992). Affected cattle with BLAD were linked to common ancestral sires that had been documented to be carriers (Jorgensen et al., 1993; Shuster et al., 1992). Several Holstein Friesian bulls were identified as BLAD carriers and the gene encoding impaired CD18 spread to many countries.

Initially lack of efficient methods for identifying such type of genetic disorders and use of semen of proven carrier bulls lead to highest level of BLAD allele frequency in Holstein in US 23% (Shuster et al., 1992), France 10% (Tainturier et al., 1995), Germany (black and white HF 13.5% & red and white HF 0.3%) (Biochard et al., 1995), Argentina HF cattle 2.88% (Poli et al., 1996), Japan HF cattle 16% to 32% (Nagahata et al., 1997), Brazil HF 2.8% (defected); 5.7% (carrier) (Ribeiro et al., 2000). This disorder had also been reported from United Kingdom (Andrews et al., 1996), Italy (Gourreau et al., 1998), Hungary (Janosa et al., 1999), Korea (Chung et al., 1997), Lithuania (Kucinsiene and Miceikiene, 2000), Uruguay (Llambi et al., 2003), Iran (Esmaelizad et al., 2002; Nassiry et al., 2005), Poland (Czarnik, et al., 1997), Denmark (Jorgensen et al., 1993), Austria (Schilcher et al., 1995) and India (Muraleedharan et al, 1999, Patel et al., 2003 & 2007, Mahdi, 2008 and Kumar, 2009) (Table 1).

Shuster et al. (1992) tested 20 calves with clinical symptoms of LAD and found that all were homozygous for the D128G allele. In addition, two calves homozygous for the D128G allele were identified during widespread DNA testing and both were subsequently found to exhibit symptoms of LAD. The carrier frequency for the D128G allele among Holstein cattle in the United States was approximately 15% among bulls and 6% among cows. All cattle with the mutant allele were related to one bull, which through the use of artificial insemination sired many calves in the 1950s and 1960s.

Kuczaka et al. (1993) examined 200 bulls being used for AI and 1500 cows, for controlling of BLAD and found 14.1% of AI bulls and 5.8% of cows were carrier.

<table>
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<tr>
<th>Country</th>
<th>Frequency of carrier</th>
<th>Reference</th>
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<tr>
<td>USA</td>
<td>23%</td>
<td>Shuster et al., 1992</td>
</tr>
<tr>
<td>France</td>
<td>10%</td>
<td>Tainturier et al., 1995</td>
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<tr>
<td>Germany</td>
<td>13.5%</td>
<td>Boichard et al., 1995</td>
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<tr>
<td>Argentina</td>
<td>2.88%</td>
<td>Poli et al., 1996</td>
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<tr>
<td>Japan</td>
<td>16-32%</td>
<td>Nagahata et al., 1997</td>
</tr>
<tr>
<td>Brazil</td>
<td>5.7%</td>
<td>Ribeiro et al., 2000</td>
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<tr>
<td>Iran</td>
<td>3.33%</td>
<td>Noruzy et al., 2005</td>
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<tr>
<td>India</td>
<td>1.33%</td>
<td>Muraleedharan et al., 1999</td>
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<tr>
<td></td>
<td>3.23%</td>
<td>Patel et al., 2007</td>
</tr>
<tr>
<td></td>
<td>7.31%*</td>
<td>Mahdi, 2008</td>
</tr>
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<td></td>
<td>21.82%*</td>
<td>Kumar, 2009</td>
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* - Selected animals were mainly paternal half sibs of bulls with an established heterozygous BLAD genotype
Screening of 4000 dairy cows and heifer in US showed that, annually more than 16000 to 20000 recessive homozygote calves were born which were ultimately removed from population due to some infections (Biochard et al., 1995). Kehrli et al. (1991) tested 815 proven bulls and found that 10 bulls were BLAD carriers.

Biochard et al. (1995) tested 359 Holstein animals who were relatives and they found that this deficiency had transmitting to next generation in Mendelian recessive manner. Mortality rate in defective animals were 50% and 100% respectively in 2 month and 1 year old calves.

Nagahata et al. (1997) tested 769 blood samples in Japan in 1994 and 1995 reported that the chances of born for BLAD are 0.16% to 0.32 % respectively. Ribeiro et al. (2000) estimated that the frequency of homozygous recessive animals was 2.8% and heterozygous animals were 5.7% in Brazil.

Bulls (146) from artificial insemination stations and cows (192) kept in different regions of Lithuania were tested. Four bulls were found to be BLAD gene carriers. As their semen was widely used for cows’ insemination in Lithuania, it resulted in 6.7% of BLAD frequency in dam population (Kucinskiene and Miceikiene, 2000).

Norouzy et al. (2005) tested 30 proven bulls in Iran and reported the frequency of carrier animals as 3.33%.

Patel et al. (2007) examined 377 HF, 334 HF crossbred, 105 Jersey and 160 other breeds and found that frequency of carriers was only 3.23% in HF and its crosses in India.

Mahdi, (2008) examined 52 HF crossbred bulls and found that frequency of carriers was 7.31% in India. Kumar, (2009) screened families of carrier bulls and found that frequency of carriers was 21.82% in his sample.

Flow cytometric analysis of leukocytes of normal, BLAD-heterozygous and BLAD-homozygous animals for the expression of CD11a, CD11b, CD11c and CD18 revealed nearly no expression in the BLAD-homozygotes and a decreased expression in most BLAD-heterozygotes compared to most of the normal animals (Cox et al., 1997).

Neutrophils from BLAD animals lack the capacity to adhere to the endothelium, a necessary step in their emigration into foci of infection. Due to the virtual absence of neutrophil-mediated host defense, animals suffer from recurrent infection of the respiratory and gastrointestinal tracts and finally succumb to infections, omphalophlebitis, leukocytosis and several febrile episodes of infection (Meylan et al., 1997; Mueller et al., 1997).

The main clinical signs were general unthriftness and leukocytosis with a high proportion of neutrophils and fibrogranulomatous perisplenitis and calcification of splenic stroma and pulmonic arteries (Agerholm et al., 1993).

DNA tests for identifying different genotypes of BLAD

In 1992 Shuster and his co workers amplified 58 bp DNA fragment including mutation site with the help of pair primers, then they have identified different genotypes with digesting the product with Taq I and Hae III restriction enzymes. This test has been carried out with different Authors and primers so the product size was different. (Tajima et al., 1993; Mirck et al., 1995; Tammen et al., 1996; Fesus et al., 1996; Kriegesmann et al., 1997; Natonek, 2000).

BLAD was detected by PCR-SSCP (Polymerase Chain Reaction- Single Strand Conformation Polymorphism Analysis) in a simple, highly accurate and high throughput manner. Results were confirmed by DNA sequencing. This study provides a more
reliable and useful method for extensive screening of BLAD and also offers a theoretical basis for molecular diagnosis in Holstein calves. Like restriction fragment length polymorphisms (RFLPs), SSCP are allelic variants of inherited, genetic traits that can be used as genetic markers. Unlike RFLP analysis, however, SSCP analysis can detect DNA polymorphisms and mutations at multiple places in DNA fragments (Orita et al., 1989). As a mutation scanning technique, though, SSCP is more often used to analyze the polymorphisms at single loci, especially when used for medical diagnosis (Sunnuck et al., 2000).

Tetra-primer ARMS-PCR (amplification refractory mutation system- Polymerase Chain Reaction) is relatively new method. It employs two primer pairs to amplify, respectively, the two different alleles of a SNP in a single PCR reaction. A computer program for designing primers was developed. Tetra-primer ARMS-PCR was combined with microplate array diagonal gel electrophoresis, gaining the advantage of high throughput for gel-based resolution of tetra-primer ARMS-PCR products. The technique was applied to analyse a number of SNPs and the results were completely consistent with those from an independent method, restriction fragment length polymorphism analysis. It is a rapid, simple, low cost and high throughput methodology for SNP genotyping (Shu et al., 2001).

Checklist for managing BLAD in a herd
1) A system should be set up for accurate recording of sire and maternal grandsire ID for all cows in the herd.
2) List or spreadsheet file should be made showing the BLAD status of the sire and maternal grandsire of each cow in the herd.
3) Selection index should be used such as Lifetime Net Merit, to identify the group of AI sires likely to be used in the herd during the next three months.
4) BLAD carrier bulls should be avoided for using on any cow whose sire or maternal grandsire is a carrier.
5) Modern genetic tools help us to identify undesirable genes and to eliminate them in a rapid and efficient manner and should be utilized.

CONCLUSION

The massive spread of genetic disorder in cattle like BLAD in recent years is caused by the extensive use of elite sires and latent heterozygous carriers. Artificial insemination accelerates the spread of undesirable recessives world-wide. However, the new methods of molecular genetics enable us to find the cause at gene level. They make it possible to detect heterozygous animals, to control the genetic health of the population and in a way, to anticipate the cumulation of recessive alleles. The first step in controlling genetic defects like BLAD is to establish pedigree records for the animals in a herd. Once this is done, it’s relatively easy to avoid mating known carrier bulls to cows whose sire or maternal grandsire is also a carrier, because virtually all AI sires will be tested. One can do this by visually inspecting pedigrees, by developing a simple spreadsheet program, or by using a computerized mating program. It’s probably unwise to “panic” and exclude all BLAD carrier bulls from a breeding program. Many bulls that carry the undesirable BLAD gene may also carry numerous other genes with positive effects on milk production, milk component percentage, udder shape and size, somatic cell count and other key traits. If one discards all of these bulls, one may end up using a somewhat mediocre group of bulls instead, just to avoid BLAD. Avoid using BLAD carrier bulls on cows whose sire or maternal grandsire are BLAD carriers.
REFERENCES