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Incidence of hereditary Citrullinemia and bovine leucocyte adhesion deficiency syndrome in Indian dairy cattle (BOS TAURUS, BOS INDICUS) and buffalo (*BUBALUS BUBALIS*) Population

(short communication)

Summary

PCR based screening of the cattle and buffaloe bulls for Bovine Leucocyte Adhesion Deficiency Syndrome (BLAD) and Citrullinemia genetic disorders were carried out. A total of 330 cattle belonging to 4 breeds and 135 buffaloes of 3 breeds were genotyped. One young Holstein bull was detected as carrier (0.67%) for mutation at codon 86 in the argininosuccinate synthetase gene locus. Two young Holstein bulls were diagnosed as heterozygous (1.33%) for the BLAD locus. So far no carriers were detected in the indigenous cattle and buffalo breeds.

Key words: cattle, buffaloes, monogenic diseases, bovine leucocyte adhesion deficiency, citrullinemia, polymerase chain reaction, DNA typing, DNA sequencing

Zusammenfassung

Titel der Arbeit: **Bedeutung der erblichen Citrullinemie und des Bovinen Leukozyten Adhäsions Defizienz Syndroms bei indischen Milchrindern *(Bos taurus, Bos Indicus)* und Büffeln *(Bubalus bubalis)*** (Kurzmitteilung)

Ein Screening der Erbdefekte Citrullinemie und Bovinen Adhäsions Defizienz wurde bei Rindern und Büffeln durchgeführt. Insgesamt wurden 330 Rinder aus 4 Rassen und 135 Büffel aus 3 Rassen genotypisiert. Ein Holstein Bullenkalb (0,67%) konnte als Anlagenträger für die Mutation im Codon 86 im Argininsuccinat Synthesegenlocus identifiziert werden. Zwei Holstein Jungbullen wurden als heterozygot (1,33%) für den BLAD Locus diagnostiziert. Bei den indischen Rinder- und Büffelrassen wurden keine Träger dieser Erbdefekte gefunden.

Schlüsselwörter: Rinder, Büffel, monogenetische Defekte, LAD, Citrullinemie, Polymerasekettenreaktion, DNA Typisierung, DNA Sequenzierung

Introduction

Over five thousand monogenic diseases are known in human beings. In animals a smaller number of genetic diseases are characterised with a known genetic basis. With the wide spread use of artificial insemination (AI) and international trading of semen and breeding animals, hereditary diseases have attained greater importance in dairy animals. Individual AI sires can be exploited economically for important genetic traits through large number of their progenies. However at times, it can also be disadvantageous, if AI bulls are carriers of a deleterious trait or a hereditary disease. Intensive use of a bull carrier for a particular genetic disease can spread the disease very rapidly in the population. Most of the genetic diseases are difficult to detect as they are inherited as recessive autosomal. It

is therefore important to screen the animals, especially the breeding bulls at an early age to avoid great economic loss to cattle breeders. Two most important monogenic diseases that affect cattle breeding are Bovine Leucocyte Adhesion Deficiency (BLAD) and Bovine Citrullinemia. The molecular basis of BLAD and Citrullinemia is described elsewhere in literature. BLAD is due to a transition of (adenine - guanine) at position 383 in the gene coding for CD18 protein resulting in deficiency of CD11b/CD18 glycoprotein complex located on the surface of neutrophil granulocytes (KEHRLI et al., 1990). Neutrophils lacking in CD11b/CD18 are unable to migrate to the site of inflammation and due to this impaired neutrophil function the animal suffer from recurrent infections.

Bovine Citrullinemia is caused by a transition of Cytosine - Thymine at codon 86 within exon 5 in the gene coding for argininosuccinate synthetase enzyme (ASAS), leading to impaired urea cycle (DENNIS et al., 1989). So far no attempt has been made to assess the degree of incidence of these genetic diseases in Indian dairy cattle and buffalo population although we import semen and animals from the countries where these diseases are known to be prevalent. Through this study we have tried to find out the occurrence of these inherited diseases in our dairy animal population.

Materials and Methods

Blood and semen samples were collected from male animals reared at farms of co-operative milk unions across the country. Breeds of cattle and buffaloes included in this study were, Holstein-Friesian (n=150), Jersey (n=50), Sahiwal (n=40), Gir (n=40), their Crossbreeds (n=50) and three buffalo breeds, Murrah (n=75), Surti (n=40) Jaffarabadi (n=20). All the animals were either bulls used for A.I. or young bulls reared for breeding purpose. DNA was prepared by following standard protocols and also by using Instagene matrix columns available from BIORAD.

Genotype analysis

The mutation at CD18 gene locus was detected on the basis of diagnostic test developed by SHUSTER et al. (1992) with minor modifications. In brief the reaction contained 100 ngs of genomic DNA, five pmoles of sense primer (5' TCC GGA GGG CCA AGG GCT A-3') and anti sense primer (5' GAG TAG GAG AGG TCC ATC AGG TAG TAC AGG - 3'), 1X PCR buffer, 200 μM dNTPs and 1.5 units of Taq DNA polymerase (Perkin-Elmer) in a 25 μl reaction volume. The PCR reaction conditions were as follows : 94^0 C for 25 seconds; 68^0C for 20 second and 72^0C for 20 seconds for 30 cycles with an additional 10 minutes at 72^0C. In each case 10 μls of amplified product was digested with both *Hae* III and *Taq* I restriction enzymes in separate tubes to completion and analysed on 4% agarose gels. Amplification of ASAS locus and detection of mutation at codon 86 was done by PCR followed by restriction digestion of amplified products. The reaction contained 20 pmoles of sense (5`GGC CAG GGA CCG TGT TCA TTG AGG ACA TC 3`) and antisense primers (5`TTC CTG GGA CCC CGT GAG ACA CAT ACT TG 3`)

(GRUPE et al., 1996), 1x PCR buffer, 200 µM dNTP's and 1 unit of Taq DNA polymerase (Boeringher Manheim) in 25 µl reaction volume. The PCR reaction was carried out at 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds for 30 cycles with an additional 72°C for 10 minutes. The amplified products was digested in 20 µl volume with 5 units of *Ava* II restriction enzyme for 3 hrs and analysed on 3% NuSieve agarose gel containing ethidium bromide at a final concentration of 0.5 µg/ml.

Sequencing of PCR products

Amplified products which were identified as carriers were directly sequenced using PCR product sequencing kit (Amersham-USB). PCR products from carrier samples for both BLAD and Cirullinemia were sequenced and samples were analysed on 6% glycerol tolerant polyacrylamide gel and autoradiographed.

Results and Discussion

The amplified 58 base pair product for CD18 locus upon *Hae* III digestion yielded 2 bands of 49 bp and 9 bp respectively for normal animals, bands of 49bp, 30bp, 19bp and 9bp respectively for carrier animals which were further confirmed by *Taq* I digestion which yielded bands of 32bp and 26 bp respectively for normal animals and three bands of 58 bp, 32bp and 26 bp respectively for carrier animals indicating loss of restriction site for *Taq* I (Fig. 1). The finer bands were further confirmed through polyacrylamide gel electrophoresis with restriction endonuclease treated PCR products generated using the same primers that were end labelled with γP^{32} ATP prior to PCR amplifcation. The PCR - RFLP of ASAS locus product were 103bp and 82bp for normal animals and 185bp, 103bp and 82bp for carrier animals indicating the transitional mutation of C-T at codon 86 of ASAS gene (GRUPE et al., 1996). The results of PCR - RFLP are shown in Figure 2.

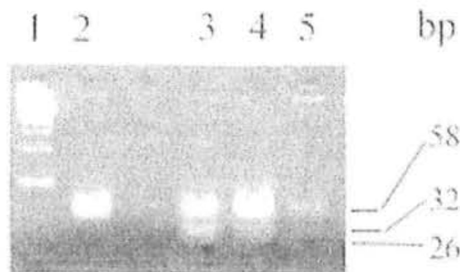

Fig. 1: Electrophoretogram of Taq I digested PCR product generated by amplification of genomic DANN using CD 18 locus specific primers. Lane 1: 100bp marker, Lane 2: 58pb band of homozygous BLAD animal indicating loss of restriction site; 3 - 4: 58, 32 and 26 bp bands of heterozygous carrier; 5: undigested 58 bp PCR product (Elektrophoretogram von mit TaqI gespaltenen PCR-Produkten, die auf der Amplifikation genomischer DNA von spezifischen Primern für den CD18 Locus entstanden sind. Lane 1: 100bp Längenmarker, Lane 2: 58pb langes Fragment eines homozygoten BLAD Tieres, das den Verlust des Restriktionsortes zeigt. Lanes 3 - 4: 58, 32 und 26 bp lange Fragmente von heterozygoten Trägertieren, Lane 5: 58bp langes, ungespaltenes PCR-Produkt)

Fig. 2: Ava II restriction digestion pattern of PCR product generated by amplification of genomic DNA using ASAS locus specific primers. Lane 7: 185, 103 and 82 bp bands of heterozygous Citrullinemia carrier animal. Lanes 1,2,4, 5 and 6 : 103 and 82 bp bands of normal animal; lane 3 : 100 bp marker (Ava II Spaltungsbild eines PCR-Produktes aus genomischer DNA unter Verwendung von ASAS-Genort spezifischen Primern. Lane 1 und 3: 185, 103 und 82 bp Fragmente von heterozygoten Citrullinemie Trägertieren. Lanes 2, 5 und 6: 103bp und 82 bp lange Fragmente von normalen Tieren. Lane 4: 100bp Längenmarker)

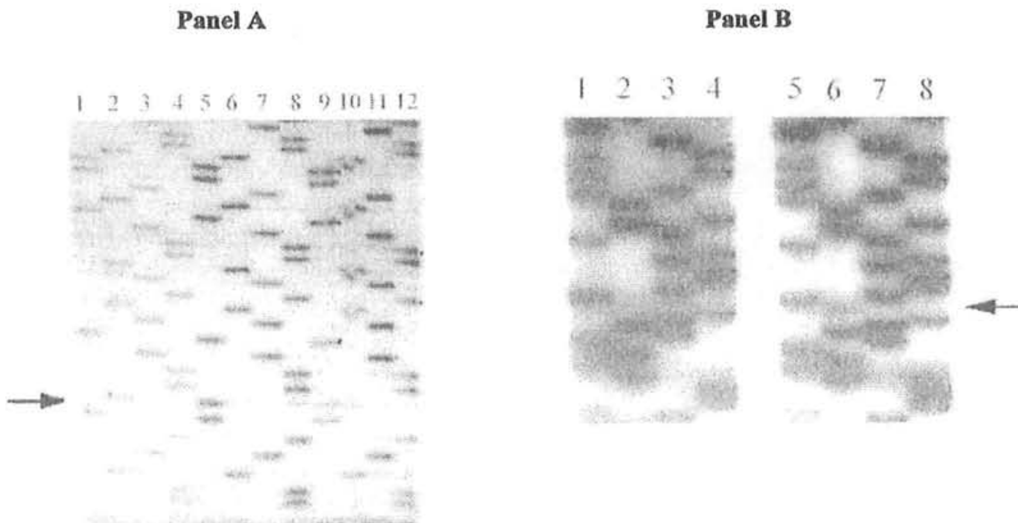

Fig. 3: Partial sequence of CD18 and ASAS locus showing the transition mutation (Teilsequenz des CD18 und des ASAS Locus die die Transitionsmutation zeigen)

Panel A: Partial sequence of CD 18 locus; Lane 1 - 4: GATC sequence of PCR product generated from normal animal; 5 - 8: GATC sequence of homozygous BLAD PCR product indicating the transition of G to A (marked with an arrow); 9 - 12: GATC sequence of heterozygous BLAD PCR product (Panel A: Teilsequenz des CD18 Locus, Lane 1-4: GATC Sequenz der PCR-Produkte eines normalen Tieres. Lane 5-8: GATC Sequenz eines homozygoten BLAD PCR-Produktes, das die Transition von G nach A zeigt (markiert durch einen Pfeil); Lanes 9-12: GATC-Sequenz eines heterozygoten BLAD PCR-Produktes)

Panel B: Partial sequence of ASAS locus PCR product generated from carrier and normal animals; Lane 1 - 4:CTAG sequence of homozygous normal animal; 5 - 8: CTAG sequence of heterozygous carrier animal showing the mutation of C to T (marked with an arrow) (Panel B: Teilsequenz des ASAS Locus von einem normalen und einem Anlagenträger. Lanes 1-4: CTAG-Sequenz eines heterozygoten Trägertieres das die Mutation von C nach T zeigt (markiert mit einem Pfeil))

All the animals belonging to three breeds of buffaloes were found to be normal for both BLAD and ASAS locus. Among the cattle two young Holstein bulls had a mutant allele for CD18 locus indicating their carrier status. Animals belonging to other cattle breeds were normal for this locus. Among the cattle breeds one Holstein bull calf was tested heterozygous (carrier) for ASAS locus. All other animals of various cattle and buffaloe breeds were normal. The results are shown in the Table. Heterozygous (carrier) status of animals tested were confirmed by direct sequencing of PCR products. The partial sequence is shown in Figure 3.

Table
Results of genotyping for Citrullinemia and Bovine Leucocyte Adhesion Deficiency (BLAD) in different cattle and buffalo breeds in India (Ergebnisse der Citrullinemie und der Bovinen Leukozyten Adhäsions Defizienz (BLAD) Typisierung in verschiedenen Rinder- und Büffelrassen Indiens)

Species	**Cattle**					**Buffalo**		
Breed	Holstein Friesian	Jersey	Sahiwal	Gir	crossbreds	Murrah	Surti	Jaffarbadi
Number of animals analysed	150	50	40	40	50	75	40	20
Citrullinemia (%)								
homozygous normal	99.33	100	100	100	100	100	100	100
heterozygous mutant	0.67	0	0	0	0	0	0	0
homozygous mutant	0	0	0	0	0	0	0	0
BLAD (%)								
homozygous normal	98.67	100	100	100	100	100	100	100
heterozygous mutant	1.33	0	0	0	0	0	0	0
homozygous mutant	0	0	0	0	0	0	0	0

The Holstein bull identified as carrier was imported from Australia where this mutation is reportedly wide spread. HEALY et al. (1991) reported that 50% of Australian National Friesian herds and 30% of A.I. bulls in A.I. centres are descendants of Linmack Kriss King (LMKK) which was a carrier for Citrullinemia. Although no proper pedigree was available for tracing the origin of mutation in the carrier animal we identified, it is possible that this animal could be a 3rd or 4th generation descendant of LMKK. In other countries like USA and Germany, the incidence of the Citrulliemia is very low (ROBINSON et al., 1993; GRUPE et al., 1996). Two animals heterozygous for BLAD were born through A.I. with the semen imported to this country. We have started screening the breeding bull and bull calves routinely with the objective of diagnosis and prevention of the spread of BLAD and Citrullinemia in our dairy animal population.

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