SCREENING FOR GENETIC DISORDERS IN INDIAN MURRAH AND SURTI BUFFALO *(BUBALUS BUBALIS)* BULLS

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ABSTRACT

Genetic disorders constitute a menacing threat whose consequences often become evident only after several generations of breeding, when short-term, low cost solutions are no longer possible. The present study involved screening of 135 buffaloes viz. Murrah buffalo (n=106) and Surti buffalo (n=29) bulls for autosomal recessive genetic disorders such as Bovine Leukocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Bovine Citrullinemia (BC) and Factor XI Deficiency (FXID) using PCR based techniques. Genomic DNA was extracted from blood by High Salt Method and the fragments of genes of interest were amplified by PCR technique. The amplified PCR products were digested with *TaqI, AvaI and AvaII* restriction enzymes for BLAD, DUMPS, and BC, respectively. Bulls were screened for FXID based on PCR conformation. The screening of Murrah and Surti bulls revealed that none of the buffaloes screened were carrier for BLAD, DUMPS, BC and FXID.

Keywords: genetic disorders, BLAD, DUMPS, bovine Citrullinemia, FXID, Buffaloes, PCR-RFLP

INTRODUCTION

Buffalo, a triple purpose animal, provides milk, meat and mechanical power to mankind. Buffalo was originated from Asian wild buffalo which has been domesticated since pre-historic times in Asia particularly in Indo-Pak subcontinent. Riverine buffalo, *Bubalus bubalis*, was domesticated nearly 5000 years ago in Iran, Iraq and Indo-Pak subcontinent, whereas domestication of swamp buffalo, *Bubalus carabensis*, took place in China and other part of Southeast Asia after 1000 years (Bruford *et al*., 2003). India has 57% of the world's buffalo population. Riverine buffaloes contribute enormously to the rural economy and have adapted to the existing ecosystem over the years and have gained eminence as an important dairy animal in Indian subcontinent. The importance of this species to the Indian dairy industry is immense; buffalo (*Bubalus bubalis*) constitute 35% of the bovine population in India but they contribute more than 52.6% to the total milk production (BAHS, 2010).

Recessive autosomal genetic disorders are found at very low frequencies in livestock, but they have a disproportionate economic impact on livestock agriculture. Most of the genetic diseases in domesticated species are inherited as autosomal recessive traits, and carriers generally give no outward indications, the undesirable trait can be spread widely and covertly. Artificial insemination

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has, since its inception, revolutionised the buffalo breeding programme there is, however, an ever-present danger in its widespread application; undiagnosed genetic defects might spread rapidly through the use of a carrier bull's semen.It has become necessary to screen all animals to minimize the risk of spreading these diseases to next generation. Understanding the molecular basis of a genetic defect makes it possible to detect carriers directly at the DNA level and more important, early in the animal's life. The diagnosis method based on PCR and PCR-RFLP based analysis is more reliable and useful method for extensive screening for genetic disorder.

Some of the known genetic disorders in bovines are Bovine Leukocyte Adhesion Deficiency (BLAD), Bovine Citrullinemia (BC), Deficiency of Uridine Monophosphate Synthase (DUMPS), Complex Vertebral Malformation (CVM) and Factor XI deficiency (FXID). BLAD is an autosomal recessive genetic disorder which results in death of homozygous animals. It is characterized by reduced level of expression of the adhesion molecules on neutrophils called as β–integrins, a complex of CD 11/CD 18 family of proteins that helps the neutrophils to migrate to the site of inflammation (Nagahata *et al*., 1987). BLAD is caused mainly due to point mutation (A→G) at the position 383 of CD 18 gene located on the first chromosome of bovine, which causes an aspartic acid to glycine substitution at amino acid 128 (D128G) in glycoprotein.

There is also existence of silent point mutation (C→T) at position 775 in the CD 18 gene (Shuster *et al*., 1992). Afflicted animals show series of severe symptoms, including impaired wound healing and stunted growth, persistent marked neutrophilia, chronic diarrhea, ulcers on oral mucous membranes, chronic pneumonia, gingivitis, loss of teeth, high fever and other infections (Kherli *et al*., 1990; Shuster *et al*., 1992). The enzymatic deficiency for Uridine-5-monophosphate synthase (DUMPS) is recessive genetic disorder that interferes with the biosynthesis of pyrimidines. UMPS is mapped in bovine chromosome 1 which catalyzes the conversion of orotic acid into Uridine monophoshate, precursor for all other pyrimidines and normal constituent of the milk in cow and other ruminants.(Robinson *et al*., 1993). DUMPS is caused by point mutation of C→G in codon 405 of exon5 (Harlizius *et al*., 1996). Animals homozygous for DUMPS do not survive to birth and usually die early in gestation. The embryos abort approximately 40 days after conception, leading to repeated breeding problems (Lee *et al*., 2002). Citrullinemia is a rare metabolic disorder characterized by serious neurologic symptoms in newborn calves (Harper *et al.*, 1986). It is caused by C86G transition within exon 5 in the gene coding for arginino succinate synthetase (ASS) enzyme which leads to error of urea metabolism. This conversion results in a truncated peptide product (85 amino acids long rather than the normal 412 amino acids) that lacks enzyme activity. This mutation also eliminates a restriction site for the enzyme *AvaII* which will cut the normal gene but not cleave the mutant gene (Dennis *et al*., 1989). It is characterized by high levels of citrulline, and more seriously, of ammonia in plasma.

After birth, these calves, display neurological problems that become progressively worse. Depression is observable within a day; followed by unsteady gait, head pressing, aimless wandering, apparent blindness, collapse, convulsions, and death within one week (Healy *et al*., 1990; Harper *et al*., 1986). FXI Deficiency is an autosomal recessive bleeding disorder which causes increased susceptibility to infectious

diseases, mastitis, metritis and pneumonia, low calving and survival rates (Liptrap *et al*., 1995). The mutation consists of insertion of poly adenine tract (76bp AT(A)28TAAAG(A)26GGAAATAAT AATTCA) into exon 12 of FXI on chromosome 27 which introduces a premature stop codon leading to synthesis of non functional protein (Marron *et al*., 2004). A very low incidence of BLAD were observed in earlier studies carried out in Holstein population in Brazil, Iran, Turkey and India (Nagahata *et al*., 1997; Ribeiro *et al*., 2000; Rahimi *et al*., 2006; Meydan *et al*., 2007; Arpita *et al*., 2012). Earlier studies showed that all the buffaloes screened were normal for BLAD (Muraleedharan *et al*., 1999; Rajesh *et al*., 2007). DUMPS carrier cases have been reported in different countries in America and Europe in cattle (Citek and Blahova, 2004). Very low incidence of genetic disorders for DUMPS were observed among cattle population in Poland (Kaminski *et al*., 2005), India (Rajesh *et al*., 2006), Turkey (Akyuz *et al*., 2009; Meydan *et al*., 2010) and Iran (Rahimi *et al*., 2006). None of the males were either carrier or affected for Bovine Citrullinemia in cattle population (Meydan *et al*., 2010; Rajesh *et al*., 2006; Citek *et al*., 2006) and for FXID (Cyrus *et al*., 2011; Saeed *et al*., 2012). Earlier workers reported that all the buffaloes screened for Bovine Citrullinemia (Muraleedharan *et al*., 1999) and FXID (Saeed *et al*., 2012; Rajesh *et al*., 2007) were normal and none of them were carriers.

MATERIALS AND METHODS

Animals and extraction of DNA

India possesses the best River milk breeds in Asia which include Murrah, Nili-Ravi, Surti and Jaffarabadi, which originated from the north-western states of India and have a high potential for milk and fat production apart from their use as a work animal and as a supplementary stock for use as meat production (Sethi, 2003). A total of 135 buffalo bulls belonging to Murrah (n=106) and Surti (n=29) breeds maintained at different State frozen semen stations in Karnataka, India were utilized for the study. Blood sample (10 ml) was collected aseptically from each bull by jugular veinipuncture into vacutainer tubes containing EDTA and was stored at 4^{o}C till further use. Within 24 h after collection of blood, genomic DNA was extracted by High Salt Method as described by Miller *et al*. (1988) with minor modifications. Agarose gel electrophoresis and spectrophotometric methods were used to determine quality, quantity and purity of DNA. The samples showing an optical density (OD) ratio (260 nm/280 nm) of between 1.8 to 2.0 were stored at -20^{o}C and used for further analysis and diluted to 100 ng$^{-\mu l}$ for PCR analysis work.

Screening of genetic disorders based on PCR–RFLP technique

The fragments of genes of interest were amplified by PCR technique. Primers, PCR product size, annealing temperature and restriction enzymes (RE) used in the PCR for identification of Bovine Leukocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Bovine Citrullinemia (BC) and Factor XI Deficiency (FXID) are presented in Table 1. DNA was amplified with initial denaturation at 94^{o}C for 5 minutes, followed by 35 cycles consisting of denaturation at 94^{o}C for 1 minute, specific annealing temperature for 1 minute, extension at 72^{o}C for 1 minute, with final extension 72^{o}C for 5 minutes. Genotypes were determined using agarose gel electrophoresis (1.5%) stained with ethidium bromide. The genotypes for BLAD,

Table 1. Primers, PCR product sizes, Annealing temperature and restriction enzymes (RE) used for identification of Bovine Leukocyte Adhesion Deficiency (BLAD), Deficiency of Uridine Monophosphate Synthase (DUMPS), Bovine Citrullinaemia (BC) and Factor XI Deficiency (FXID).

Genetic disorder	**Primer sequence (5'→3')**	**PCR product size (bp)**	**Annealing temperature (˚C)**	**Restriction enzyme used**	**Reference**
BLAD	F: CCTTCCGGAGGGCCAAGGGCT R: CTCGGTGATGCCATTGAGGGC	136 bp	57°C	*TaqI* at 65^0C for 5 h	Citek *et al.,* 2006
DUMPS	F: AGGGTCTTAGTGGAGCAGGT R: GGCTTACCTCCTGCTTCTAACTG	282 bp	54°C	*AvaI* at 37^0C for overnight	Designed based on ENSEMBL number ENSBTAG00000013727
BC	F:GGCCAGGGACCGTGTTCATTGAGGAC ATC R:TTCCTGGGACCCCGTGAGACACATACTTG	198 bp	57°C	*AvaII* at 37^0C for overnight	Grupe S, *et al.,* 1996
FXID	F: CCCACTGGCTAGGAATCGTT R: CAAGGCAATGTCATATCCAC	320 bp	55°C	Direct PCR assay	Marron *et al.,* 2004

DUMPS and BC were identified by using PCR-RFLP analysis. The PCR products were digested with *TaqI*, *AvaI* and *AvaII* restriction enzymes for BLAD, DUMPS and BC, respectively. The FXID genotypes were detected by PCR analysis by running the amplified product on 1.5% agarose gel. 10 µl of PCR products of BLAD, DUMPS and BC were digested with particular enzyme. The digested products were separated on 3% agarose gel and analyzed by visualizing the gels under Gel doc system (Bio-Rad, USA). Representative samples were sequenced and sequences were confirmed by Amnion Bioscience Private Ltd, Bangalore, India.

RESULTS AND DISCUSSION

A total of 135 buffaloes belonging to Murrah (n=106) and Surti (n=29) breed were screened for autosomal recessive genetic disorders viz. BLAD, DUMPS and BC using PCR-RFLP technique and FXID was screened by PCR conformation. The primers used in the study successfully amplified the DNA fragments of 136 bp for BLAD, 282 bp for DUMPS, 198 bp for BC and 320 bp for FXID. In case of BLAD, carrier animal produces three fragments of 136 bp, 108 bp and 28 bp, affected animal shows one band of 136 bp. In the present study the amplified product of CD 18 gene upon digestion by *TaqI*, yielded two fragments of 108 bp and 28 bp in all buffalo bulls showing normal homozygote animals hence no animal were found to be either affected or carrier for BLAD (Figure 1). The amplified product of uridine monophosphate synthase upon digestion by *AvaI*, yielded two bands of 213 bp and 69 bp for normal animals. Carrier animals produce three bands of (282 bp, 213 bp and 69 bp) and affected animals produce single band of 282 bp. Among the screened buffalo, all animals were found to be normal in case of DUMPS (Figure 2). To detect the point mutation in gene coding for argininosuccinate synthase (ASS), the amplified product upon digestion by *AvaII* yielded two bands of 109 and 89 bp for normal animals (Figure 3). Carrier animals produce three bands of 198, 109 and 89 bp and affected produce single band of 198 bp. None of the buffaloes screened were neither affected nor carrier for BC. In FXID, unaffected animals produce a fragment of 244 bp, carrier animals produce two fragments of 320 bp and 244 bp and affected animal produce a fragment of 320 bp. All the screened buffaloes were found to be negative for FXID (Figure 4).

The spread of genetic disorders in cattle and buffalo have been increased in recent years. In the present study all the screened animals were found to be normal and none of them were carrier for BLAD, DUMPS, BC and FXID. Similar results were observed in earlier studies carried out in buffalo for BLAD (Muraleedharan *et al*., 1999; Rajesh *et al*., 2007), Bovine Citrullinemia (Muraleedharan *et al*., 1999) and FXID (Saeed *et al*., 2012; Rajesh *et al*., 2007). Similar results of very low or lack of incidence of BLAD were observed in earlier studies carried out for Holstein population (Nagahata *et al*., 1997; Ribeiro *et al*., 2000; Rahimi *et al*., 2006; Meydan *et al*., 2007; Arpita *et al*., 2012). DUMPS allele has been reported in different countries in America and Europe (Citek and Blahova, 2004), Poland (Kaminski *et al*., 2005), India (Rajesh *et al*., 2006), Turkey (Akyuz *et al*., 2009; Meydan *et al*., 2010) and Iran (Rahimi *et al*., 2006). Previous studies in cattle for Bovine Citrullinemia showed none of the males to be either carrier or affected (Meydan *et al*., 2010; Rajesh *et al*., 2006; Citek *et al*., 2006) and for FXID (Cyrus *et al*., 2011; Saeed *et al*., 2012). The present study suggests that the

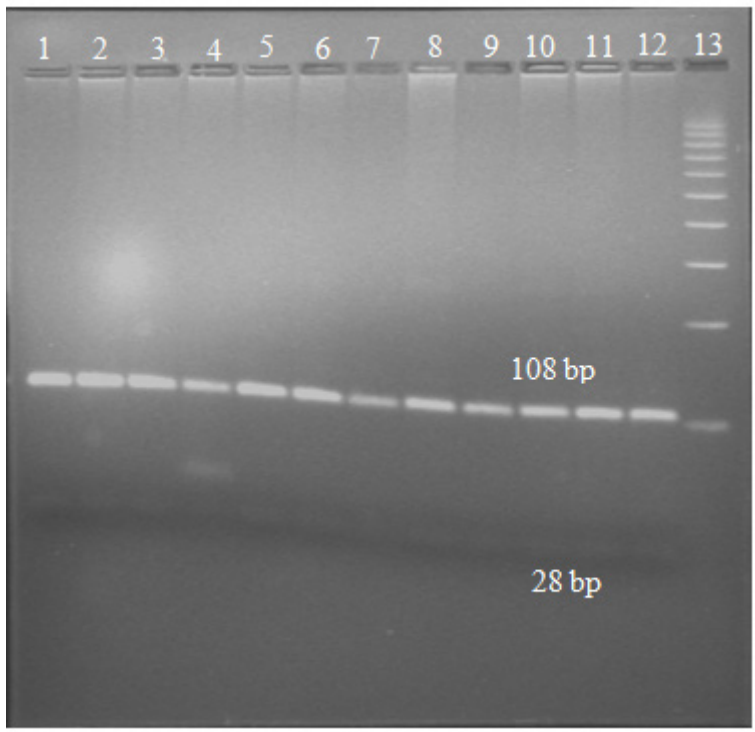

Figure 1. PCR-RFLP result of CD18 gene: Lane 1 to 12 showing normal homozygote two fragments, 108bp and 28bp (not visible in gel); Lane 13 - 100 bp DNA Marker.

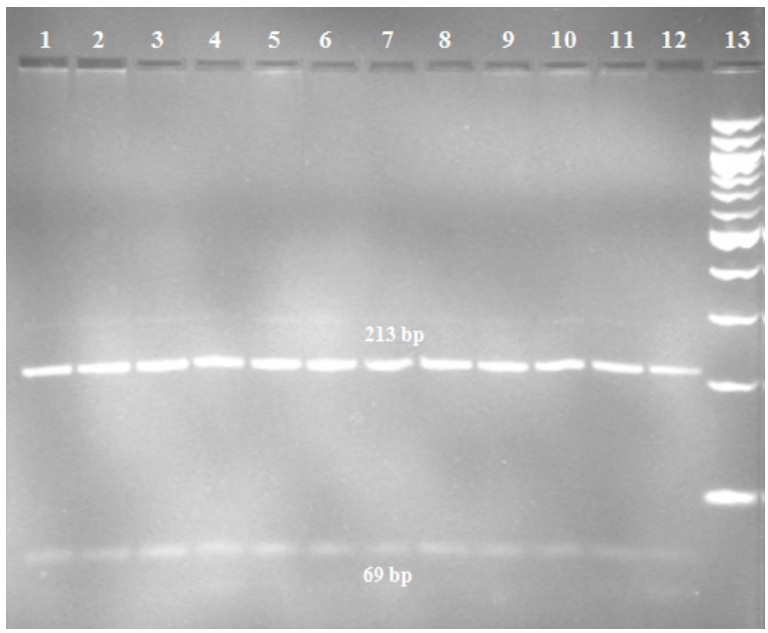

Figure 2. PCR-RFLP result of gene coding for uridine monophosphate synthase: Lane 1 to 12 normal homozygote animal showing two fragments of 213bp and 69bp; Lane 13 - 100bp DNA Marker.

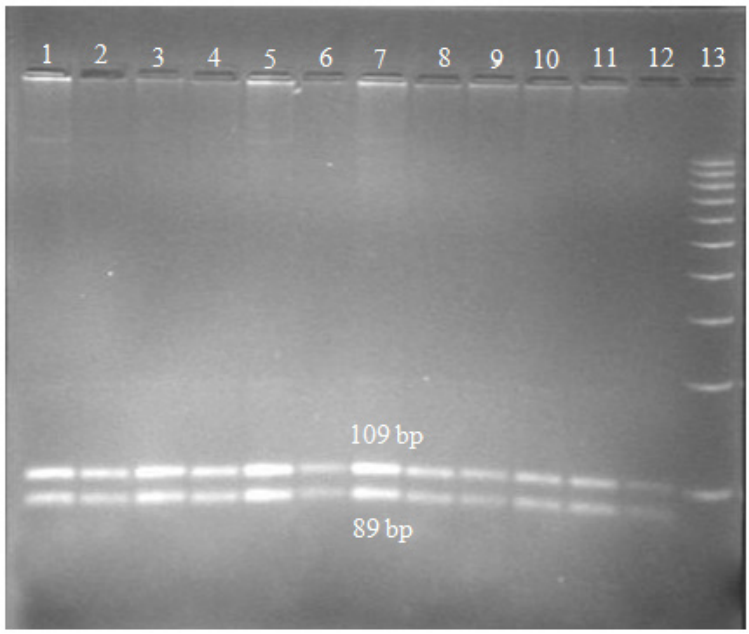

Figure 3. PCR-RFLP result of Arginosuccinate synthase gene: Lane 1 to 12 normal homozygote showing two fragments, 109bp and 89bp; Lane 13 - 100bp DNA Marker.

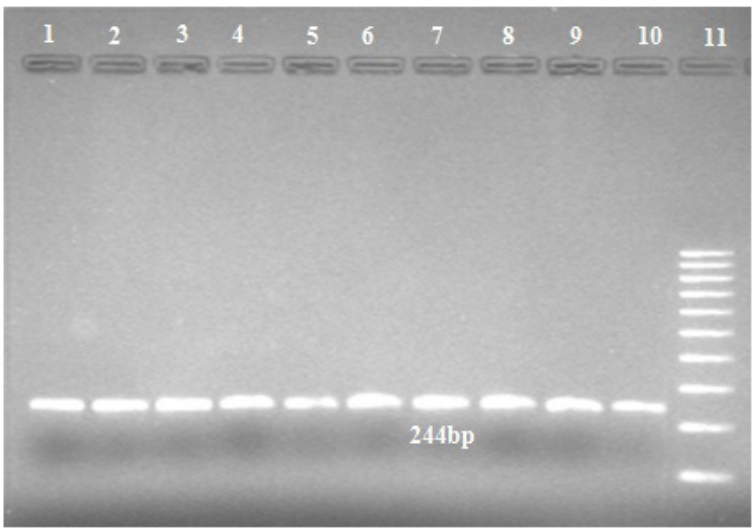

Figure 4. Polymerase Chain Reaction (PCR) genotyping of FXID: Lane 1 to 10 showing product size 244bp, normal homozygote genotype; Lane 11 - 100bp DNA Marker.

PCR and PCR-RFLP based technique could be employed as an efficient technique for screening of various genetic disorders for identification of carriers or affected animals before using them in breeding programmes to minimize the spread of defective allele. Molecular markers are identified as powerful tool which helps in diagnosis of genetic disorders at a very early stage. Hence it is required to establish the screening methods allowing breeders to test their animals to minimize the spread of disease in a population.

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