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RESEARCH ARTICLE

Novel mutation in UMPS gene leads to false positive result of DUMPS (genetic disorder) in buffaloes: need for gene sequencing before confirming results of RFLP in new species

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Abstract. Deficiency of uridine monophosphate synthase (DUMPS) is a lethal genetic disorder associated with early embryonic mortality. Murrah and Mehsana male buffaloes (n = 594) were screened for DUMPS by PCR-RFLP technique. A few Murrah buffalo male calves were found to be carriers of DUMPS in RFLP, which has not been reported earlier. On the Sanger sequencing, a novel A to G substitution mutation was identified in *Ava*I restriction recognition site of UMPS gene in buffaloes. This mutation hinders digestion of DNA by *Ava*I which leds to false positive results for DUMPS carrier in RFLP. The results indicated that genome sequencing must be performed before confirming results of RFLP in any new species. All the buffaloes that were tested had only wild-type genotype in exon 5 for DUMPS specific allele.

Keywords. buffalo; deficiency of uridine monophosphate synthase; false positive; lethal; genetic disorder.

Introduction

Genetic disorders pose serious economic implications to the dairy industry whose consequences often become evident only after several generations of breeding. Chances of propagation of genetic disorders through carrier bulls are very high in artificial insemination programme. A large number of genetic disorders have been recognized in animals which lead to embryonic loss, reduced fertility, foetal mortality, reduced immunity, physical deformities etc. These genetic disorders are often breed/species-specific and inherited, affecting all kinds of farm animals. Some of these genetic disorders are due to single-nucleotide polymorphisms (SNP) but others are due to changes in larger haplotypes up to a few Mbp.

Deficiency of uridine monophosphate synthase (DUMPS) is an autosomal recessive disorder mainly affecting Holstein Friesian cattle and its crossbreds characterized by cytosine to thymine (C→T) mutation at position 1247 of uridine monophosphate synthase (GenBank accession no. X65125.1; Clark *et al.* 2016). Uridine monophosphate synthase (UMPS) has a key role in the pyrimidine nucleotide (DNA and RNA) synthesis, which is essential for normal growth and development of several ruminant and nonruminant species (Healy and Shanks 1987). The embryos with homozygous recessive alleles die in early days of pregnancy due to disruption in embryo development process. However heterozygous animal do survive and live a normal life. Bulls having carrier state may have reduced conception rate (Robinson *et al.* 1993; Lee *et al.* 2002).

Early reports have shown absence of mutant allele for economically important genetic disorders in Indian buffalo breeds (Muraleedharan *et al.* 1999; Ramesha *et al.* 2017). However, Centre for Analysis and Learning in Livestock and Food (CALF), NDDB tests the DNA samples of buffalo bulls and bull calves for DUMPS by RFLP (Schwenger *et al.* 1993). During such routine screening, a few Murrah buffalo were found to be carriers for DUMPS in RFLP analysis. This prompted further investigation to verify and confirm the presence of this mutation in buffaloes.

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Materials and methods

Sample collection and DNA extraction

DNA samples from buffaloes in farmers' herds located in Haryana, Punjab, Uttar Pradesh and Gujarat and stored in CALF lab repository were used for the study. DNA samples belonged to 594 buffaloes: Mehsana (76) and Murrah (518). The purified DNA quantity and quality was estimated by using spectrophotometer and agarose gel electrophoresis prior to PCR amplification.

Genotyping of DUMPS by PCR-RFLP

To detect the mutation in the gene coding for UMPS, a polymerase chain reaction (PCR), followed by restriction analysis (PCR-RFLP) was carried out. The genomic DNA was amplified to 282 bp fragment by performing PCR reaction with a set of primers: F (5′-AGGGTCTTAGTG-GAGCAGGT-3′) and R (5′-GGCTTACCTCCTGCTTC-TAACTG-3′) (Schwenger *et al.* 1993). The PCR reaction was set to 25 μL volume. The amplification reaction conditions included initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 45 s, annealing of primers at 62°C for 30 s and extension at 72°C for 30 s, followed by final extension at 72°C for 10 min. The amplified PCR product of desired size was analysed on 2% w/v agarose (low EEO) gel and stained with ethidium bromide and visualized under UV transilluminator. Further, the PCR product was subjected to digestion overnight by using *Ava*I restriction enzyme in 1× reaction buffer at 37°C. The digested products were visualized on 2.5% agarose gel and the results were documented by Gel Doc system. Digestion of this amplified PCR product of uridine monophosphate synthase with *Ava*I yielded two bands of 213 and 69 bp in wild-type DNA without DUMPS mutation; DNA with mutation remains undigested (282 bp) and hence heterozygous animal shall exhibit all the three fragments, i.e. 282 bp, 213 bp and 69 bp. Animals homozygous for DUMPS produce only one band of 282 bp.

Sequencing of UMPS gene

Based on the PCR-RFLP results, five representative samples heterozygous for the DUMPS gene and two normal animals were sequenced for further confirmation. To amplify the target, PCR was performed as mentioned above. The amplified PCR product was purified using Qiagen PCR purification kit (Qiagen, Germany). The sequencing of UMPS gene was performed at Omics facility, Veterinary College, Anand. The sequences obtained were processed with MEGA 7 software and submitted to NCBI GenBank (MT188661). The nucleotide sequences were compared and aligned with the reference sequences of DUMPS coding sequence (NCBI GenBank KF829950.1) using Clustal X multiple alignment program.

Results and discussion

DUMPS is due to lethal allele affecting animal productivity during early embryonic period. In the present study, 594 Mehsana and Murrah buffaloes were screened for the presence of DUMPS mutation. The amplified PCR products (282 bp) upon digestion by *Ava*I enzyme yields two bands of 213 and 69 bp in the animals without DUMPS mutation, DNA strand with mutation remains undigested (282 bp) and hence, heterozygous animal exhibit all the three fragments, i.e. 282 bp, 213 bp and 69 bp (figure 1). Among the 594 buffaloes screened, 62 Murrah buffaloes were identified to be heterozygous carriers for DUMPS indicated by three bands. Most of the carrier animals were related to one Murrah buffalo bull. This specified bull had conception rate of 40.6% which was normal under field performance recording programmes. All the Mehsana bulls were found to be normal in RFLP analysis.

DUMPS has not been reported in buffaloes until now (Rajesh *et al.* 2006; Ramesha *et al.* 2017). Hence, to confirm further, a few representative DNA samples (both normal and carrier in RFLP analysis) were sequenced. The alignment of the nucleotide sequences of representative samples with the reference sequences revealed a novel substitution mutation (G→A) within recognition sequence of *Ava*I restriction enzyme, i.e. 5' C|YCGRG…3' with Y being C or T (NCBI GenBank accession number MT188661). The presence of the mutation (CCC**G**AG to CCC**A**AG) prevented *Ava*I cleaving the DNA. This had resulted in false positive for DUMPS in PCR-RFLP. The results also showed that

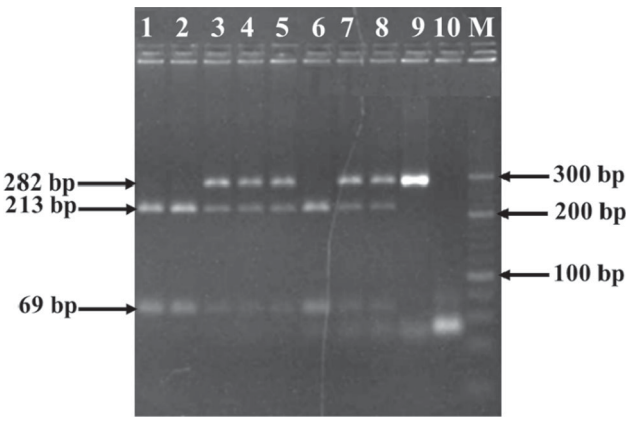

Figure 1.. PCR-RFLP for detection of DUMPS in buffaloes. M, molecular size marker (100 bp DNA ladder); (1,2,6) homozygous UMPS allele wild genotype; (3–5,7,8) suspected heterozygotes for DUMPS. (10) negative control for DUMPS; (9) homozygous positive control for DUMPS.

frequency of novel mutant is low and present only in heterozygous condition. The sequenced region of UMPS gene in buffalo has a per cent identity of 97.44% with *Bos indicus* x *B. taurus* (XM_027536431.1), *B. mutus* (XM_005893401.2) and *Bison bison* (XM_010834280.1) indicating that the gene is highly conserved among Bovines.

In the present study, a novel A to G substitution mutation was identified in Murrah buffaloes in *Ava*I recognition site in UMPS gene. This mutation may be a species specific one which leads to change in amino acid from glutamine to arginine (Q→R). The impact of the mutation on uridine monophosphate synthase enzyme synthesis and its phenotype expression should be studied. The results indicated that genome sequencing must be performed before confirming the results of RFLP in detection of mutation in a new species/population.

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