



SHORT COMMUNICATION

Screening of Cattle Breeds for 17bp Deletion in a Gene Causing Ehlers-Danlos Syndrome, Type VII (Dermatosparaxia)

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ABSTRACT

Most of autosomal disorders are breed specific except a few like Ehlers-Danlos syndrome, type VII (Dermatosparaxia) which is reported in Holstein, Belgium Blue, Charolais, Hereford, Simmentaler cattle etc. Ehlers-Danlos Syndrome is a heritable collagen dysplasia causing skin extensibility and fragility in animals and humans. The disease caused by different mutations like single base pair change, and 3 base pair change followed by a 17bp deletion in the beginning of the sequence of the gene coding for the enzyme procollagen 1N-Proteinase (pNPI) in different breeds of cattle. Clinical signs and severity of condition vary between species. The disorder characterized by tearing of skin, hyperextensibility and fragility of skin. For the present study, 142 blood samples from Holsteins, Holstein crossbreds, Red Knadhari, Khillar and Gaolao breeds were collected in EDTA vacutainer tubes. The DNA was extracted using PCR to detect 17bp deletion. Out of 142 samples, 131 samples were amplified by PCR. The study revealed that none of cattle considered for the investigation, exhibited 17bp deletion in the functional gene. However, it needs to investigate all kind of mutations in a candidate gene in a large population to assert the presence or absence of Ehlers-Danlos syndrome, type VII (Dermatosparaxia). Since the sample size was limited, it is advisable to cover more population and study the occurrence of other mutations causing the symptoms of diseases in different breeds of cattle in India.

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INTRODUCTION

Most of the autosomal recessive genetic diseases are breed specific except few and one of them rarely occurring genetic disorder is Dermatosparaxia which affects many breeds. Dermatosparaxia is a heritable collagen dysplasia, which causes the tearing of skin hyperextensibility and fragility of skin, where as in other species, joint laxity and blood vessel abnormalities have also been described (Colige *et al.*, 1999). The genetic disorders in cattle may occur at very low to low and carriers look normal therefore, are usually not detected. Dermatosparaxia has been known in humans since the 17th century, and was first described as a collagen abnormality by Ehlers from Denmark and Danlos from France (Van Leuven, 1987). This autosomal recessive genetic defect has been reported in humans, dogs, mink, cattle, sheep, cats and horses (Van Leuven, 1987), but in South Africa only in humans and white Dorper sheep (Van Halderen

and Green, 1988). Clinical signs and severity of the condition vary between species.

There are two kind of Ehlers-Danlos Syndrome in cattle; i) Ehlers-Danlos syndrome (EDS) or Dermatosparaxia which is characterized by articular hypermobility, skin hyperextensibility, and tissue fragility affecting skin, ligaments, joints, blood vessels, and internal organs, observed in Holstein, Belgium Blue, Charolais, Hereford, Simmental and crossbred cattle (Scott and Miller, 2003), and Drakensberger cattle in South Africa (Holm *et al.*, 2008). Tajima *et al.*, (1999) reported that this disorder in Holsteins is due to a mis-sense mutation (G254A) in the gene for dermatan sulfate proteoglycan, resulting in a serine-to-asparagine substitution in the serine-glycine repeat portion of the peptide, which is the binding portion of the peptide. The peptide is now called epiphygan, and its gene is called EPYC. The gene is mapped on 5q21 of *Bos taurus*. ii) Ehlers-Danlos syndrome, type VII (Dermatosparaxia) in *Bos taurus* is due to a mutation in

the gene for the enzyme procollagen I amino proteinase, which is the enzyme responsible for removing "surplus" amino acids from the N-terminal end of procollagen-I molecules. Colige *et al.* (1999) showed that the disorder in cattle is due to 17-bp deletion in a candidate gene, now called ADA metalloproteinase with thrombospondin type I motif, 2 (ADAMTS2). The molecular cause of the condition was shown to be a defect in the procollagen protease enzyme that is an endopeptidase involved in the processing of collagen, leading to abnormal orientation of collagen fibrils within collagen fibres and resulting weak fibres (Nuytinck *et al.*, 2000). The present article is based on screening of cattle breeds for 17bp deletion in the gene causing Dermatosparaxis in cattle.

MATERIALS AND METHODS

The blood samples were collected in EDTA-vacutainer blood collecting tubes from 142 animals mainly bulls of various breeds for the present study. The breeds which are used for present study were mainly Holstein Friesian (HF) (*Bos taurus*), HF Crossbred (*Bos taurus* x *Bos indicus*), Red Kandhari, Khillar, and a few from Gaolao (*Bos indicus*). DNA was isolated by the rapid methods using cell lysis buffer, nucleus lysis buffer, phenol: chloroform: isoamyl alcohol (25:24:1) which was finally precipitated in 70% alcohol.

Quantity and quality of DNA was determined by Agarose gel electrophoresis and Nano-drop. As described by Colige *et al.*, (1999), 101 bp DNA fragment was amplified by Polymerase chain reaction (PCR), which was set by adding forward primer (5'-ACCCGCGTGGAGCCCCTGCT-3') and reverse primer (5'CCAGCCCATCGCAGTTGCTGAG -3'). The PCR mix contained 1X PCR buffer, 1.5 mM MgCl₂, 10 mM dNTPs (Kapa Bio systems), 5 pM each of forward and reverse primer (Bioserve), 5 Unit *Taq* DNA polymerase, 160ng genomic DNA and distilled water to make a final volume of 20µl. The PCR reaction included the following steps: Predenaturation for 5 minutes at 95°C followed by 35 cycles of 45 seconds at 95°C, 45 seconds at 56°C, 60 seconds at 72°C and final extension for 7 min at 72°C for utilization of extra dNTPs in mixture. Master mix for PCR to amplify 101bp contains following components in a final volume of 20 µl. After PCR, tubes were removed and 6 µl of PCR product as mixed with 2 µl of 6X loading dye. The PCR product with dye was loaded on 2% Agarose gel along with 100bp and 10bp DNA ladder for visualization.

RESULTS AND DISCUSSION

In order to screen Ehlers-Danlos syndrome genetic disease in cattle breeds DNA was extracted from blood cells for PCR analysis. The quality and quantity of DNA was assessed before PCR. The DNA concentration was estimated by Nano-drop method. In some of samples the concentration of DNA (ng/µl) was very low but PCR product was amplified except few samples. However, some of samples were showing sufficient DNA concentration in sample Khillar, HF but could not be amplified. Some of samples mainly with lower concentration of DNA showed very less amplification.

The quality and quantity of most of samples were good. Precisely, out of 142 samples, due to whatsoever reasons, 11 could not be amplified therefore, 131 samples exhibited normal band of 101bp which are shown in electrophoretogram picture (Fig 1). The normal PCR products of 131 samples reveals that there is no mutation (deletion of 17bp) from a gene for the enzyme procollagen I amino proteinase causing Ehlers-Danlos Syndrome (EDS) or Dermatosparaxis. Otherwise heterozygous (carrier) would have exhibited PCR products of 101 and 84bp.

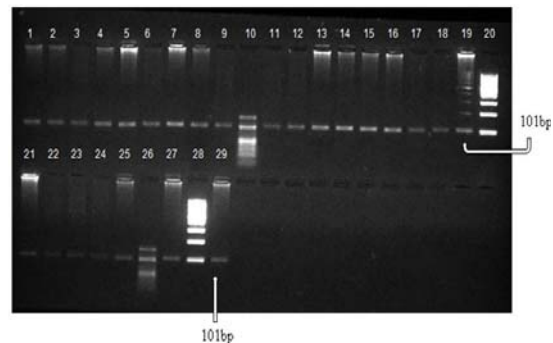


Fig. 1: Electrophoretogram (2% agarose) of PCR product generated by amplification of genomic DNA using Dermatosparaxis (EDS) specific primers. Lane # 10, 26 & 20, 28: 50bp and 100bp DNA Ladders respectively. (Thermo Scientific) Lane # 1-29: PCR product of 101bp of normal cattle.

This was first attempt in India to investigate Ehlers-Danlos syndrome (EDS) or Dermatosparaxis in cattle breeds of India. However, none of animal exhibited 17bp deletion in a candidate gene. However, the disease/carrier for the defective gene was observed in a few countries. The collagen defects condition was also observed in other species like goat, sheep (Norwegian Dala, Border Leicester-Southdown, Finnish-Merino cross, Romney, White Dorper), pigs (Large White-Essex cross), horses (Quarter horse, Arabian cross), rabbits (New Zealand white), cats (Himalayan and domestic shorthair), mink, and dogs (Van Leuven, 1987; Van Halderen and Green, 1988). The mode of inheritance of the condition is recessive in all species except domestic shorthair cats wherein it is dominant. Since the sample size was limited, it is advisable to cover more population and study the occurrence of other mutations causing the symptoms of diseases in different breeds of cattle in India. It should also be taken up in routine screening in India as other breed specific autosomal recessive genetic syndromes like; Bovine Leukocyte adhesion deficiency (BLAD) (Roy *et al.*, 2012), Citrullinaemia (Gaur *et al.*, 2012), Factor XI deficiency (Patel *et al.*, 2007), Deficiency of Uridine Monophosphate Synthase (Gaur *et al.*, 2013) and Complex Vertebral Malformation (CVM) (Kotikalapudi *et al.*, 2013) are regularly screened to remove heterozygous (carrier) animals from ongoing breeding programmes.

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