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Polymorphism study of Fec X gene in indigenous sheep of Assam

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Abstract
The present study was conducted to check the polymorphism of Fec X gene in the indigenous sheep breeds of Assam. A total of 50 unrelated sheep covering Barpeta, Bongaigaon, Goalpara, Hekera and Kamrup districts of Assam was taken into consideration for the research work. Blood samples were collected and genomic DNA was isolated. PCR amplification of the DNA samples produced 141 bp amplified product. The PCR-RFLP technique was employed using Hinf1 restriction enzyme which resulted into a single type of banding pattern with two fragments of 110 and 31 bp in all the samples studied. Fec X gene was found to be monomorphic in the studied population.

Keywords: indigenous sheep, Fec X gene or BMP15, PCR-RFLP, Hinf1

Introduction
Sheep occupy an important place in the agricultural economy of India. It is mainly reared by small, marginal, landless and nomadic farmers throughout the country. Sheep provide a wide array of raw materials. According to 20th Livestock census of India [1] possess 74.26 million sheep with a growth of 14.13%. Apart from the improvement programmes running under the government for the betterment of sheep and other livestock in the country, the upward trend in sheep population compared to the previous can also be attributed to the factor that they are drought resistant animals and survive even on the dried blades of grass and leaves. They have short generation intervals, higher rates of prolificacy, and marketing of sheep meat products is far easier and thereby making it more preferable for the poor farmers to get engaged into sheep rearing.

Among the north eastern states Assam has maximum number of Sheep accounting for 3.25 Lakhs (Twentieth Livestock census India 2019). Around 50.95 thousands of sheep are being slaughtered in Assam every year [2]. In Assam the sheep population is distributed throughout the lower Assam covering mainly Barpeta, Bongaigaon, Dhubri, Goalpara, Kamprup and Nalbari districts. Assam does not possess any registered breed of sheep so far but the indigenous breed of sheep is reared mainly for meat purpose. They are predominantly maintained on natural vegetation on uncultivated lands and waste lands. It becomes an inseparable component of mixed farming system in view of the existing socio-economic conditions of the state. Rearing of sheep plays an importing role in supporting the poor farmers economically. The people of the state are mostly non-vegetarian and prefer the mutton over other meat. An average of 50.95 thousand sheep is being slaughtered in Assam every year [2].

The improvement of reproduction traits in sheep breeding has taken immense concern, and that is how increasing the litter size can direct into a large profit for the farmers. Prolificacy and litter size are essential economic traits of sheep breeding, and they are controlled by a set of genes called the Fecundity (Fec) genes [3]. Majority of the sheep breeds have one or two lambs at each lambing [4]. The mutations occurring naturally in the bone morphogenetic protein 15 (BMP15) genes are linked to increased ovulation rate and litter size [5]. The ovine BMP15 gene is carried by the X chromosome and is also known as FecX gene (Fecundity X gene) [5]. The identification of several mutations in the genes affecting prolificacy in sheep have given an opportunity in characterizing large numbers of ovine breeds by examining the polymorphism of these genes. This research work has been conducted to access the polymorphism of Fec X gene in the indigenous sheep breeds of Assam.

Materials and Methods

The sheep from Barpeta, Bongaigaon, Goalpara, Hekera and Kamrup districts of Assam were taken as the research animal. Around 3 ml of blood samples were aseptically collected from the jugular vein of the animal into vacutainers containing EDTA (2.7%). A total of 50 unrelated sheep covering Barpeta, Bongaigaon, Goalpara, Hekera and Kamrup districts of Assam was taken into consideration for the research work. The blood samples were taken into the laboratory in ice boxes right after collection and stored at -20º Celsius.

Extraction of DNA: Genomic DNA was extracted by Phenol Chloroform extraction Procedure [6] with a slight modification in the process. After the extraction of genomic DNA, its purity was assessed by UV spectrophotometer (Nanodrop Spectrophotometer, by checking the optical density (OD) value at 260 and 280 nm. The DNA samples having OD ratio (269 nm/280nm) 1.7 to 1.9 were utilised further. The quality of the DNA was checked by 0.8% horizontal submarine agarose gel electrophoresis and the concentration was checked in spectrophotometer. The DNA samples with a concentration of 50-90µg/ml were used for the experiment.

PCR and PCR-RFLP: The amplification of DNA was done for a total volume of 50 µl with a reaction mixture containing nuclease free water (20 µl), PCR master mix (26 µl), DNA template (2 µl) and forward and reverse primers (1 µl each). The primers were to obtain a stock primer solution of 100 pmol/ µl and further diluted to a working concentration of 10 pmol / µl.

Primer set suggested by Hanrahan *et al.* (2004) [3] to amplify FecX gene was used:

- Forward: 5'CAC TGT CTT CTT GTT ACT GTA TTT CAA TGA GAC3'
- Reverse: 5'GAT GCA ATA CTG CCT GCT TG-3'

Table 1: PCR reaction cycling conditions used were as follows

Steps	Temperature	Time
Initial denaturation	94 °C	4 minutes
Denaturation	94 °C	45 seconds
Annealing	66 °C	45 seconds
Extension	72 °C	50 seconds
Repeat step 1 to 35 cycles		
Final extension	72 °C	10 minutes
Hold at 4 °C forever		

After amplification of the PCR products they were then subjected to digestion by the restriction enzyme (RE). The restriction enzyme chosen for carrying out the PCR-RFLP was

Gene	Restriction Enzyme	Source	Restriction Site
FecX	**Hinf1**	Haemophilus influenzae	5'G↓ANTC3' 3'CTNA↑G5'

A total of 20 µl reaction volume was prepared containing 1 µl nuclease free water, 15µl of PCR products, 2 µl of buffer and 2 µl of restriction enzyme (*Hinf1*) and kept for an incubation period of 6 hours at 37 °C. To examine the RFLP products 4% agarose gel electrophoresis was run for 2 hour at 85 Volt, along with 50 bp ladder. The bands were visualized in the gel documentation system.

Sequencing and analysis of molecular data: Few samples were sequenced by outsourcing at Molbiogen (Apical Scientific Sequencing) after purification of respective PCR product. The statistical analysis of molecular data was carried out by statistical software Bioedit.

Results and Discussions

The PCR amplification of FecX gene produced amplicon size of 141 bp (Fig.1) [7] and [8] have reported similar size for the amplified product in Moghani and Ghezel Sheep of Iran and in Garole sheep, respectively. It was confirmed by 1.5% agarose gel electrophoresis ran for one hour at 90V along with 50 bp ladder. The gel was visualized by gel documentation system.

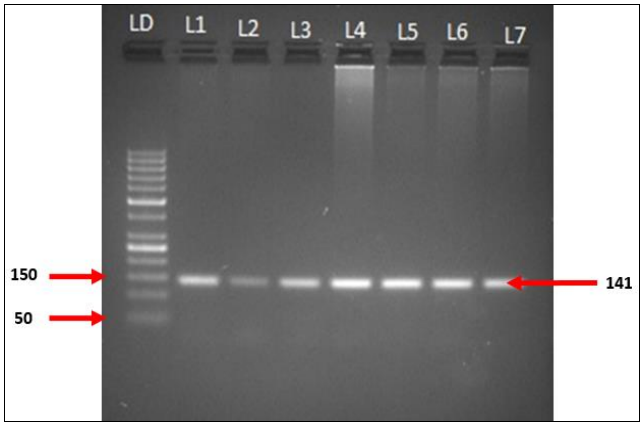

Fig 1: L1-L7: PCR AMPLICON (141bp) OF *Fec X* GENE; LD: 50bp LADDER

The digestion of amplified product with restriction enzyme HinfI yielded a single type of banding pattern with two fragments of 110 and 31 bp (Fig. 2). This can arbitrarily be designated as AB genotype. The results can be compared with [9] who also observed absence of polymorphism in the BMP15 gene loci in Barki, Ossimi and Rahmani sheep breeds of Egypt [10]. Also revealed AB genotype in case of Kenguri sheep. Similar reports have been presented by [11] in Volgograd breed of Russia and by [12] in Watish Sudanese desert sheep.

On the contrary to the present finding, by [13] in Hu and Han sheep breed of China; [14] in small tailed Han sheep; [15] in Indian Kendrapada sheep and [16] in Deccani and Nellore sheep have found three genotypes for the FecX gene.

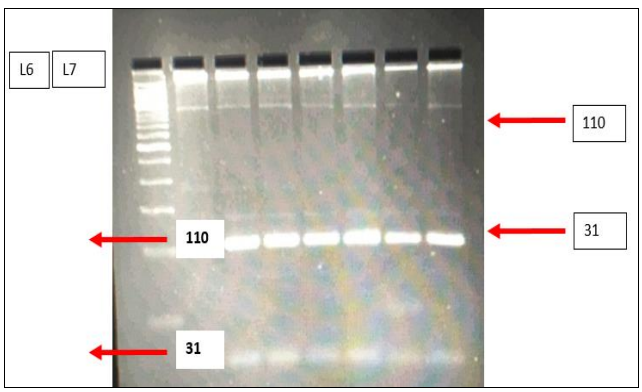

Fig 2: L1-L7: PCR-RFLP Products (110 and 31 bp); M: 50bp LADDER

CCGCCTAAAAAGAGAAGGGTCTTTTTCTGTAACTCTTTCAGGCCTTTAGGGAG
AGGTTTGGTCTTCTGAACACTCTGAGTCTCATTGAAATACAGTAACAAGAAG
ACAGTGAG

Fig 3: Fec X gene sequence of AB genotype and highlighted area is the *HinfI* restriction site

Conclusion

Population of sheep under study exhibited no polymorphism for the Fec X gene and shown to possess only one kind of genotype represented as AB. However, further study may be undertaken to confirm it in a substantially large population. Also, more research planning and work should be made in examining other fecundity gene which might be accountable for the prolificacy of the indigenous sheep of Assam.

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References

1. Twentieth Livestock Census, Department of Animal Husbandry & Dairying, Ministry of Fisheries, Animal Husbandry & Dairying.
2. Basic Animal Husbandry Statistics (BAHS). Department of Animal Husbandry and Dairying (DAHD), Government of India 2019.
3. Hanrahan JP, Gregan SM, Mulsant P, Mullen M, Davis GH, Powell R *et al.* Mutations in the genes for oocyte-derived growth factors GDF9 and BMP15 are associated with both increased ovulation rate and sterility in Cambridge and Belclare sheep (*Ovis aries*). Biology of Reproduction 2004;70(4):900-909.
4. Fugarti NM. A review of the effects of the Booroola gene (FecB) on sheep production. Small Ruminant Research 2009;85:75-84.
5. Lassoued N, Benkhlil Z, Woloszyn F, Rejeb A, Aouina M, Rekik M *et al.* FecX ^{Bar} a Novel BMP15 mutation responsible for prolificacy and female sterility in Tunisian Barbarine Sheep. BMC Genet 2017;18:43.
6. Sambrook J, Russel DW Molecular cloning: A laboratory manual, 3rd Edn, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York 2001.
7. Barzegari A, Atashpaz S, Ghabili K, Nemati Z, Rustaei M, Azarbaijani R. Polymorphisms in GDF9 and BMP15 associated with fertility and ovulation rate in Moghani and Ghezel sheep in Iran. Reproduction in domestic animals 2010;45(4):666-669.
8. Kumar R, Taraphder S, Sahoo A, Das P, Paul R, Raja D. Molecular characterization of exon-2 of bone morphogenetic protein-15 (BMP-15) gene in prolific garole sheep using PCR-SSCP method. Indian Journal of Animal Health 2015;54(2):115-122.
9. Barakat IA, Salem LM, Daoud NM, Khalil WK, Mahrous KF. Genetic polymorphism of candidate genes for fecundity traits in Egyptian sheep breeds. Biomedical Research 2017;28(2):851-857.
10. Asharani A, Appannavar M, Yathish, MH, Hussain M, Kasaralikar V, Suranagi M. Molecular Variants of FecB and BMP15 Fecundity Genes in Sheep (*Ovis aries*). International Journal of Livestock Research 2018;8(8):185-195.
11. Getmantseva L, Bakoev N, Shirokova N, Kolosova M, Bakoev S, Kolosov A *et al.* Effect of the GDF9 gene on the weight of lambs at birth. Bulgarian Journal of Agricultural Science 2019;25(1):153-157.
12. Mohamed SEI, Ahmed RM, Jawasreh KI, Salih MAM, Abdelhalim DM, Abdelgadir AW *et al.* Genetic polymorphism of fecundity genes in Watish Sudanese desert sheep. Veterinary World 2020;13(4):614-621.
13. Davis GH, Balakrishnan L, Ross IK, Wilson T, Galloway SM, Lumsden BM *et al.* Investigation of Booroola (Fec B) and Inverdale (FecXI) mutations in 21 prolific breeds and strains of sheep sampled in 31 countries. Animal Reproduction Science 2006;92(1-2):87-96.
14. Zhang C, Geng L, Liu Z, Fu Z, Feng M, Gong Y. Polymorphic study of FecXG, FecGH and FecB mutations in four domestic sheep breeds in the lower Yellow river valley of China. Journal of Animal and Veterinary Advances 2011;10(17):2198-2201.
15. Dash S, Maity A, Bisoi PC, Palai TK, Polley S, Mukherjee A *et al.* Coexistence of polymorphism in fecundity genes BMPR1B and GDF9 of Indian Kendrapada sheep. Exploratory Animal and Medical Research 2017;7(1):33-38.
16. Praveena K, Ramana DBV, Pankaj PK. Booroola Gene (Fec B) Polymorphism and its Liaison with Litter Size in Indigenous Sheep Breeds of Telangana, India. Journal of Animal Research 2017;7(2):227-231.