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# Molecular Detection of Cellular Prion Protein in Brain Tissues of Black Bengal Goats in Bangladesh

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## Abstract

The cellular prion protein (PrPC) is a membrane-bound glycoprotein mainly present in the central nervous system which is necessary for the establishment and further evolution of prion disease in human and animals. The aim of the present study was to investigate the PrPC protein in brain tissues of black Bengal goat. Fifteen brain tissues were collected from different slaughter houses of three districts (Mymensingh, Manikgonj and Netrokona) of Bangladesh during January to February, 2011. The PrPC protein was detected in the brain tissues of black Bengal goats using polymerase chain reaction. The result showed all positive (100%) of the amplified samples. The standardized PCR could be used for detection of PrPC protein in different tissues of animals and humans. Sequencing of PrP gene in the black Bengal goats for the risk assessment of scrapie is needed for further study. To our knowledge, detection of PrPC protein in the brain tissues of indigenous goats is the first time in Bangladesh.



## 1. Introduction

The cellular prion protein (PrPC) is a one kind of sialoglycoprotein (Haraguchi et al., 1989) and found throughout the body even in physically healthy people and animals. The PrPC is bound to the external surface of cells by a glycoinositol phospholipid (GPI) anchor (Stahl et al., 1987). The PrPC is encoded by a chromosomal gene, and its pathological forms known as scrapie isoform (PrPSc) found in all aspects of the prion diseases in human and animals. PrPC is present in tissues of the central nervous system, but also distributed in non-neuronal tissues of sheep, the natural host of scrapie. A prion protein is an infectious agent composed a misfolded form of protein. Prion protein also contains nucleic acids (DNA, RNA, or both) along with protein components like all other infectious agents. The normal form of the prion protein is called PrPC, while the infectious form is called PrPSc- the C denotes 'cellular' or 'common' PrP, while the Sc denotes 'scrapie', one type of prion disease occurs in sheep (Priola et al., 2003).

The normal PrPC is a glycosylphosphatidylinositol-anchored glycoprotein that is predominantly expressed in the brain tissues of human and animals (Prusiner, 1998; Weissmann and Flechsig, 2003). The causative agent of prion diseases is the protease-resistant misfolded PrPSc. Prion diseases are neurodegenerative disorders i.e. Scrapie in sheep and goats which, together with bovine spongiform encephalopathy (BSE) in cattle and Creutzfeldt–Jakob disease (CJD) in humans, belong to the group of transmissible spongiform

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encephalopathies (TSEs) (Prusiner, 1998; Pappasavva-Stylianou). In all of these neurodegenerative disorders, exposure of nerve cells PrPC converts to PrPSc in aggregated deposits. All known prion diseases mainly affect the structure of the brain or other neural tissues and all are currently untreatable and ultimate fate is death (Prusiner, 1998).

It is now well established that PrPC and PrPSc are involved in the development and progression of various forms of neurodegenerative prion diseases. The protein PrPC is highly expressed in the central nervous system especially in neurons and glial cells, and also present in non-brain cells, such as immune cells or epithelial and endothelial cells. Identification of the physiological functions of PrPC in these different cell types thus appears crucial for understanding the progression of prion diseases. Though some studies have been conducted in different countries in different species. So far we know caprine scrapie has not yet been observed in Bangladesh and no one tried to detect PrPC from the brain tissues of indigenous Black Bengal Goats in Bangladesh. Considering the above facts, the present research has been designed to understand the molecular standardization of PCR for PrPC from the brain tissues of indigenous Black Bengal goats in Bangladesh.

## 2. Materials and Methods

### 2.1. Sample Collection

A total of 15 samples (brain tissues of black Bengal goat) were collected from different slaughter houses of three districts (Mymensingh, Manikgonj and Netrokona) of Bangladesh during January to February, 2011. All the samples were preserved at -80°C in the Molecular Laboratory, Department of Pathology, Bangladesh Agricultural University, Mymensingh for detection of PrPC gene. Before collecting the samples all instruments were sterilized properly.

### 2.2. DNA Extraction

DNA was extracted from brain tissues using WizardR Genomic DNA Purification Kit (Promega Corporation. 2800 Woods Hollow Road. Madison,USA) according to manufacturer instructions. Purity and quality of extracted DNA was checked on 1% agarose gels and quantification was measured using spectrophotometer UV absorption at 260/280 nm in Central Laboratory, Bangladesh Agricultural University, Mymensingh. Brain tissues were grinded in liquid nitrogen using a mortar and pestle. After grinding, approximately 20mg of ground tissue was transferred to a 1.5mL micro-centrifuge tube containing 600µL of Lysis solution. Lysate was incubated at 80°C for 5 min, gently pipetting was done during incubation. 3µL of RNase solution was added to the lysate and mixed by inverting the tubes for 3 times. After incubating at 37°C for 30 min, 200µL of Protein precipitation solution was added. Then the sample was centrifuged (13,000 x g, 3min) and supernatant containing DNA was removed and transferred in isopropanol. After gentle mixing, solution was centrifuged again and ethanol was added to the sample. After centrifugation, ethanol was allowed to aspire and 50µL of nuclease free water was added. After confirmation by electrophoresis, the DNA was stored at – 20°C until use.

**Table 1**. Primers and their sequences used in the study

| Primers | Sequences (5'-3') | Number of bases | Product size | Reference |
|---|---|---|---|---|
| PRNP(F) | GCAATGTTGCTGGCATTCTG | 20 | 1204bp | Zhou et al. |
| PRNP(R) | GCTTGTCATTTCCCAGTGCT | 20 | | 2008 |

### 2.3. Amplification of DNA by PCR

To detect the PrPc protein, PCR was performed on the extracted DNA samples. PrPC gene was amplified using the previously used gene specific primers PRNP (F) GCAATGTTGCTGGCATTCTG and PRNP (R) GCTTGTCATTTCCCAGTGCT (Zhou et al., 2008) (Table 1). PCR was done by using a commercial PCR kit (PCR Master Mixture Kit, GeNei™, Banglore Genei, BDA Industrial Suburb, Peenya, India). The PCR reaction was performed on each DNA sample in a 10 µl reaction mix. Briefly, 4.8 µl of nuclease free $H_2O$, 1 µl of Taq DNA polymerase buffer (10X), 1 µl of dNTPs, 0.5 µl of each Primer, 0.2 µl of Taq DNA polymerase, and 2 µl DNA samples were mixed in PCR tube. Water instead of extracted DNA served as negative control. Total thirty PCR

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cycles were run as: initial denaturation at 95°C 5 min, denaturation 95, 40sec, annealing at 58 for 40 sec, extension at 72 for 1 min. The amplified products were then separated electrophoretically on 1% agarose gel.

## 3. Result and Discussion

To investigate the presence of DNA of cellular prion protein in the brain tissue of black Bengal goat was performed by PCR. The PrPC gene from the brain samples was amplified using PCR with previously used specific primers PRNP (F) and PRNP (R) (Zhou et al., 2008). Of the 15 brain tissues obtained from black Bengal goat were tested for prion protein, all samples (100%) were positive showing the predicted PCR amplicon size of 1204 bp (Fig.1). The PrPC is a membrane-bound glycoprotein attached to the cell surface by a glycosylphosphatidylinositol anchor. In spite of being ubiquitously distributed throughout many tissues and cell types in most mammalian species, PrPC is especially abundant in the CNS (Saeki et al., 1996; Bendheim et al., 1992 and Bolton et al., 1985). The PCR is a rapid, accurate and highly sensitive molecular detection technique to investigate genes and protein in tissues. In our study, PCR was standardized for the detection of PrPC protein from the brain tissues of indigenous black Bengal goats in Bangladesh. Detection of PrPC gene by means of PCR has been described before by many researchers (Zhou et al., 2008; Vaccari et al., 2006; O'Rourke et al., 2004; Acutis et al., 2004; Goldmann et al., 1996).

In the present study, the gene specific primer was used in PCR and successfully amplified 1204bp product from the samples. The result of the present study appeared similar with the previous findings of Zhou et al., (2008). They detected PrPC gene from non neural tissues (blood and muscles of indigenous Chinese goats), but in the present study neural tissues (brain) was used. Babar et al., (2007) also detected PrPC gene from blood samples of goat.

Immunohistochemical and Western blot analyses were used for both qualitative and quantitative detection of PrPC protein in bovine somatic tissues (Peralta and Eyestone 2009). However, in our study, the PCR is used as a qualitative approach for the presence of PrPC gene in brain tissues. Therefore, upon our results, it could be concluded that PCR method showed a reliable, and simple method to perform and economic in terms of output. It is therefore clear that the present evaluation will help in the designing of improved PCR protocols. This study could assist for attentiveness on the further studies on the PrP sequencing and analysis of PrP polymorphisms in black Bengal goats in Bangladesh for the risk assessment of Scrapie.

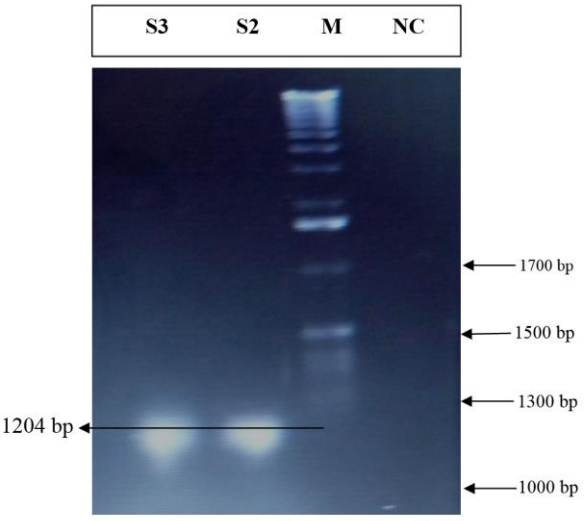


**Figure 1**: Representative amplification of the PrPC gene by PCR. M=Marker, S2=Sample-2, S3=Sample-3, NC= Negative Control without DNA. A product of expected 1204bp was successfully amplified in both the sample no. 2 and 3.

## 4. Conclusions

The aim of the present study was to investigate the PrPC protein in brain tissues of black Bengal goat. The result showed all positive of the amplified samples. The detection of PrPC protein in the brain tissues of indigenous goats is the first time in Bangladesh. The importance of the present study is to lay foundation for understanding the protein expression of PrPC in the brain tissues of goat. This is a preliminary qualitative approach for PrPC detection, further confirmation is warranted by sequencing the cellular protein in brain tissues of black Bengal goat.

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