1. INTRODUCTION

Scrapie is a contagious prion disease of sheep and goat (Prusiner, 1998). In contrast to Bovine Spongiform Encephalopathy (BSE), there is no evidence for a transmission to humans. Nevertheless, BSE is experimentally transmissible to sheep and the resulting disease can not be distinguished from scrapie (Baylis et al., 2002). Even though BSE has not been found in farm sheep yet, the possibility that BSE occurs in “scrapie” diseased sheep can not be excluded. Therefore, scrapie control programmes were implemented in many countries. Since no vaccines or therapeutic means is currently available, these programmes rely on selective breeding for scrapie resistance. Susceptibility to scrapie is largely controlled by three polymorphic amino acid positions (136, 154 and 171) of the ovine prion protein gene (Hunter, 1997) and reliable genotyping of the corresponding DNA polymorphism is required as a basis for selection decisions.

The prion diseases can affect both humans and animals, and it is invariably fatal due to the neurological damage it causes. Presently, there are no vaccines or medicines that will prevent the disease and, apart from nursing care, there are no treatments to halt or slow its inevitable course. The course of the disease is alarmingly rapid compared with other neurodegenerative disorders such as Alzheimer disease, Parkinson’s disease and motor neuron disease. However, they are rare neurodegenerative disorders affecting only 1 in a million population, which is only about 50 or so new cases in the United Kingdom each year. Despite this, remarkable attention has recently been focused on these diseases. This is due to their unique biology and also because of the fears that an epidemic of a newly recognized bovine prion disease in 1986 among domesticated animals could pose a threat to public health through dietary exposure to infected tissues (Collinge et al., 1991; Ridley and Baker, 1995). The exposure of the infected tissues may not have been considered as a problem due to modern cooking techniques and chemical treatments such as UV light and radiation. Unfortunately, this disease causing agent happens to be particularly resistant to many chemicals, resulting with little or no loss of infectivity. It could
then be said that a significant part of the population has been exposed to BSE infected food, with no known consequences. The effects could be minor with only a handful of people succumbing to the disease or the effects could be disastrous with many thousands of people falling foul of the prion disease. Therefore scientific interest has been directed at the infectivity of the prion agent between individuals and across the species barrier in relation to the incubation time, and possible treatments, cures or vaccinations.

The causative agent of scrapie is believed to be the host-encoded prion protein (PrP) (Prusiner, 1982; 1996). Genetic susceptibility to scrapie seems to be influenced by variation in the prion gene (PrP) (Goldman et al., 1994; Hunter, 1996). In sheep, variation in the PrP gene has been identified at a number of codons, but polymorphism at some codons is rare and only three codons (136, 154, and 171) have a reported linkage with the incidence of scrapie (Baylis and Goldman, 2004). The association between ovine PrP polymorphism at codons 136, 154, and 171 and scrapie susceptibility is the basis for the marker-assisted breeding for decreased scrapie susceptibility now underway in many countries (Tranulis, et al., 2002). Various PCR-based approaches have been used to determine ovine PrP sequences at codons 136, 154, and 171. These include direct DNA sequencing (Tranulis et al., 1999; Vaccari et al., 2001; Baylis et al., 2002; Sipos et al., 2002), RFLP (Hunter et al., 1993; Ikeda et al., 1995), allele-specific oligonucleotide hybridization (Ishiguro et al., 1998), and primer extension assay (Vaccari et al., 2004). However, none of these methods allows for accurate determination of the PrP haplotype, as they either do not profile more than a fragment of the gene or require sequencing, which can be confounded in heterozygous sheep. In addition, the recent identification of the novel haplotypes A136H154R171 and VRR in sheep (Kutzer et al., 2002) makes the results from the current typing methods difficult or sometimes impossible to interpret. Accordingly, a technique that is able to determine accurately and rapidly the PrP genotype of sheep is needed.

Before any association study is taken up, there is a great need to have base-line data of existing gene variants in our indigenous sheep population. In
the modern context the gene variants (SNPs and INDELs) of even un-translated region (introns, 5’ UTR, 3’ UTR, promoter regions etc.) of a gene are also found associated with expression of the traits/regulation of traits. Thus the identification of variations/variants across the entire gene in the existing gene pool is imperative. Such molecular data shall be pivotally critical in future association studies and these attempts inform us to susceptibility and resistance against the diseases through marker assisted selection (MAS). This study become more relevant in countries like India where low/zero input system of the country, where providence of vaccination, medicines and medical care are equally critical for land less farmers becomes very relevant. The ovine scrapie was first reported in Britain around 250 years ago, yet the incidences of scrapie have not been reported exceptionally. one incidence of death of four sheep in foot hills of Himalayas (Zlotnik and Katiyar, 1961). These sheep were considered to have been imported from Britain. Gupta et al., (2007) reported the prevalence of A_{136} R_{154} Q_{171} genotypes in Gaddi sheep breed from the same region, but the animals were free from clinical or sub clinical symptoms of scrapie. Keeping this report in mind, a survey of scrapie gene polymorphism in other sheep breeds of India was carried out. In this study four sheep breeds, one each from the four major sheep breed agro-climatic zones were selected for SNP identification in PrP gene exon-3 partial CD’s comprises of all the three major codons 136, 154 and 171, affecting the onset of scrapie in sheep. PCR-SSCP technique was used for the identification of novel variants with in this segment of DNA and searching of SNP in the DNA sequences.

Therefore present investigation entitled “To study the polymorphism for PrP gene and its SNP markers in sheep breeds” was undertaken with the following objectives.

1. To detect SNP markers for prion gene in sheep breed of India.

2. To study the polymorphism for PrP gene in different breeds of sheep and to identify the SNP marker for PrP gene.