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ALLELIC POLYMORPHISM AT *IGHA* GENE IN RESISTANCE TO GASTROINTESTINAL NEMATODES OF KUMAON HILL GOATS

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Abstract: The research had shown variation in immunoglobulin heavy chain (*IGHA*) constant region and its resistance to gastrointestinal parasites. The variation in caprine *IGHA* gene which encode for hinge region results in variation in flexibility of Immunoglobulin. A molecule in recognising parasitic epitopes and subsequent immune response. This result in structurally and functionally different IgA molecules and consequently lead to variation in the IgA response to parasitisation. The present study involves investigation of IGHA gene polymorphism in Kumaon hill goats. A total of 57 goats were investigated, which are exposed to natural challenge with mixed nematode parasites on the pasture. The blood samples were collected and genomic DNA was isolated. The IGHA region was amplified from genomic DNA using PCR and it has size of 316bp. The PCR product was purified and sequenced. The analysis of sequence of *IGHA* region revealed existence of two alleles (Allele 1 and 2) in these hill goats. The frequency of allele 2 is more than allele 1.

Keywords: IGHA, FEC, PCV, PCR, gene polymorphism.

Introduction

The gastrointestinal nematodes are major cause of economic losses in small ruminants. The annual treatment costs due to *H. contortus* alone have been estimated to be $103m for India (McLeod, 2004). The control of nematodes is a challenge in practice and there is search for better approaches. One of simple approaches is selecting and breeding for resistance. There has been extensive research on breed resistance to Gastro Intestinal nematodes. The resistance is associated with variation in the key genes that control immune system (Pollott et al., 2004; Strain et al., 2002; Stear et al., 2004).

Immunoglobulin A (IgA) is an antibody that plays a critical role in mucosal immunity. It acts as a first line of defense in maintenance the integrity mucosa and in the serum, it functions as a second line of defense against pathogens that may breech the epithelial boundary (Furtado *et al.,* 2004). There are a number of studies for identifying quantitative trait loci (QTL) or

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genes for resistance to GI nematodes. The QTL associated with specific IgA activity against nematode parasites is located on chromosomes 20 (Lin *et al.,* 2009). A study by Zhou et al. (2005) found the variation mainly in the hinge region of heavy chain. This variation in the hinge region of IgA may make the molecule either more or less flexible, and hence more or less able to bind antigens with range of epitope separations of any given parasite (Furtado *et al.,* 2004). This result in structurally and functionally different IgA molecules and consequently lead to variation in the IgA response to parasitisation (Zhou *et al.,* 2005; Lin *et al.,* 2009).

The present study was to explore polymorphism at IGHA region in kumaon hill goats for their resistance/resilience to gastrointestinal nematodes.

Materials and Methods

Experimental animals

A total of 57 hill goats were selected for the present study. These animals were maintained by Indian Veterinary Research Institute (IVRI), Mukteshwar (Nainital, Uttarakhand). It is located in the temperate Himalayan region of India at $29^0 28' 20'' N$, $79^0 38' 52'' E$, and has an average elevation of 2,171 metres (7,123 feet) above the mean sea level (msl).

Blood sample collection

The blood samples (5ml) were collected aseptically in plastic containers with anticoagulant (0.1% ethylene diamine tetraacetic acid (EDTA)) from the jugular vein.

Isolation of Genomic DNA

The genomic DNA was isolated using the 'Genomic DNA Purfication Kit' supplied by Qiagen using standard protocol. The isolated DNA was resolved by 0.8% agarose gel and checked for concentration and quality. The quality (ratio of A_{260}/A_{280}) and quantity ($ng\mu l^{-1}$) of genomic DNA was estimated using NanoDrop (NanoDrop 1000-Thermo Scientific Spectrophotometer, USA).

Amplification of IGHA gene

The IGHA region was amplified from genomic DNA by using primers described by Zhou *et al.* (2005) (*Table.1*). The PCR was optimized with annealing temperature 56°C. The PCR amplified product was purified for using commercial kit and sequenced with primers as described below (*Table.1*).

Table.1: Primers for the PCR amplification and sequencing of *IGHA* gene

Primer	Sequence (5′ - 3′)	Length (bp)
Primers for initial PCR		
Sense	CCA AAG CCA GCA AGA CCG T	19
Antisense	ACT CAG GAG CAG ATC CTC GA	20
Primers for Sequencing		
Sense	TAA AGG TCA GAG GGG AGG CT	20
Antisense	GAC AGG CTG GGC TCA CAG TT	20

Sequence based typing and analysis

The alleles were sequenced from both sides using automated dye cycle sequencer. The sequences obtained from the forward and reverse reactions of each gene were analysed using GENE TOOL and Lasergene program (DNASTAR, Madison, WI) software as well as visually using chromogram of corresponding gene. The nucleotide sequences were compared with sequences in the GenEMBL database using the BLAST algorithm. The heterozygous positions were assigned ambiguity codes as recommended by the IUPAC-IUB Biochemical Nomenclature Commissions. A consensus of nucleotide sequence containing ambiguity codes at heterozygous positions was generated. A neighbour joining tree was constructed on the basis of genetic distances. The new sequences described in were submitted to NCBI Gene Bank.

Results

The PCR amplicons of revealed approximately 316bp size in agarose gel electrophoresis. The PCR samples were purified and sequenced by using self designed internal primers *(Table.1).* The final PCR product was purified and sequencing of the *IGHA* region has nucleotide size of 316bp *(Fig. 1).*

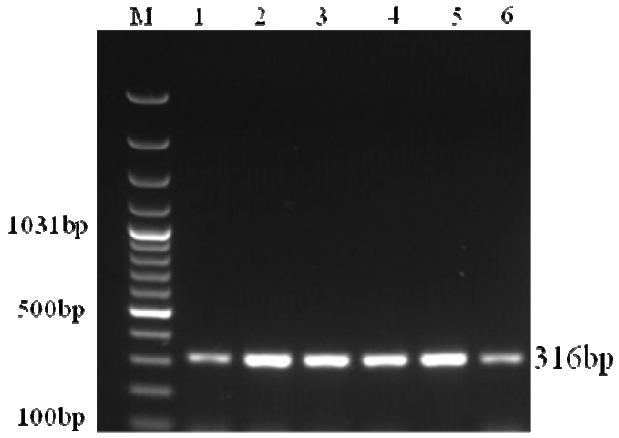

Fig.1: The amplification of *IGHA* gene showing a band at 316bp in Kumaon Hill goats
Lane M: 100bp plus DNA ladder
Lane 1-6: 316bp PCR product of IGHA region

Sequence Results

Three alleles are three known to be present in the goat, however, only two alleles are present in these hill goats of Uttarakhand and allele 3 is absent. The frequency of allele 2(89.7%) is more in hill goats than allele 1(10.3%) in table 2.

The sequence analysis revealed existence of two alleles (Allele 1 and 2) in hill goat population based on *IGHA* nucleotide. The sequencing of the *IGHA* revealed a nucleotide size of 316bp. The sequence consist of truncated exon with 1 to 42bp (exon of constant region (CH1) of heavy chain), followed by intron from 43 to 232bp, hinge region from 233 to 259bp and exon from 260 to 316bp (exon of constant region (CH2) heavy chain) (Fig.2). The main difference between allele 1 and allele 2 is single nucleotide polymorphism at 37^{th} position in exon of CH1, where cytosine (allele 1) is replaced by thiamine (allele 2) (Fig.2). At intron, only two animals revealed variations, one variation at 56^{th} position C to G (animal no.830) and five variations at 116 C to G, 119 C to G, 177 C to G, 215 C to G and 219 C to G (animal no. 855).

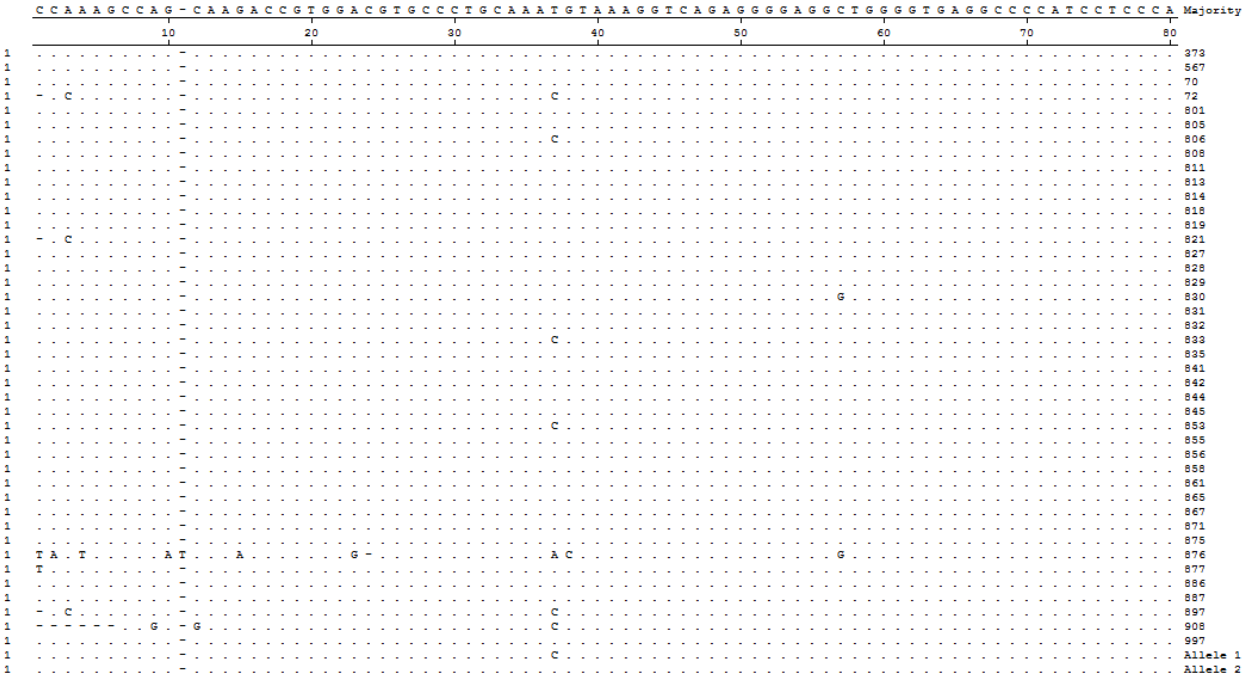

Fig.2: The nucleotide sequence alignment of IGHA with published alleles

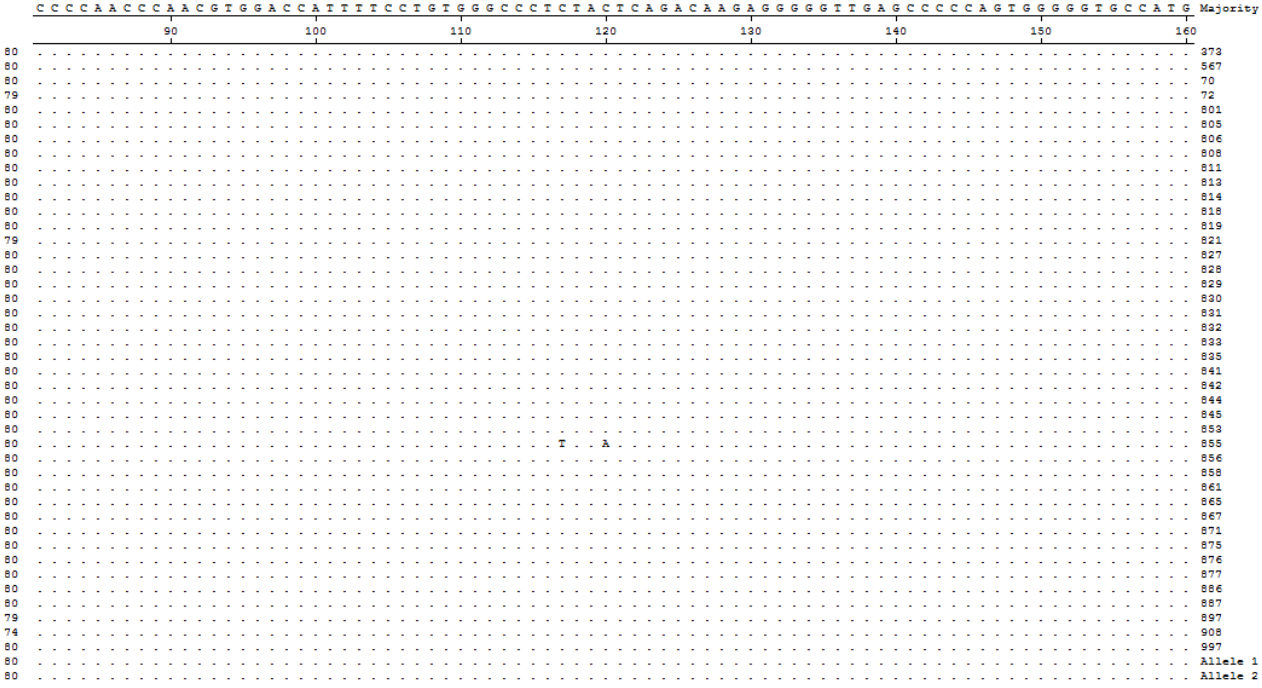

Fig.2: The nucleotide sequence alignment of IGHA with published alleles (cont...)

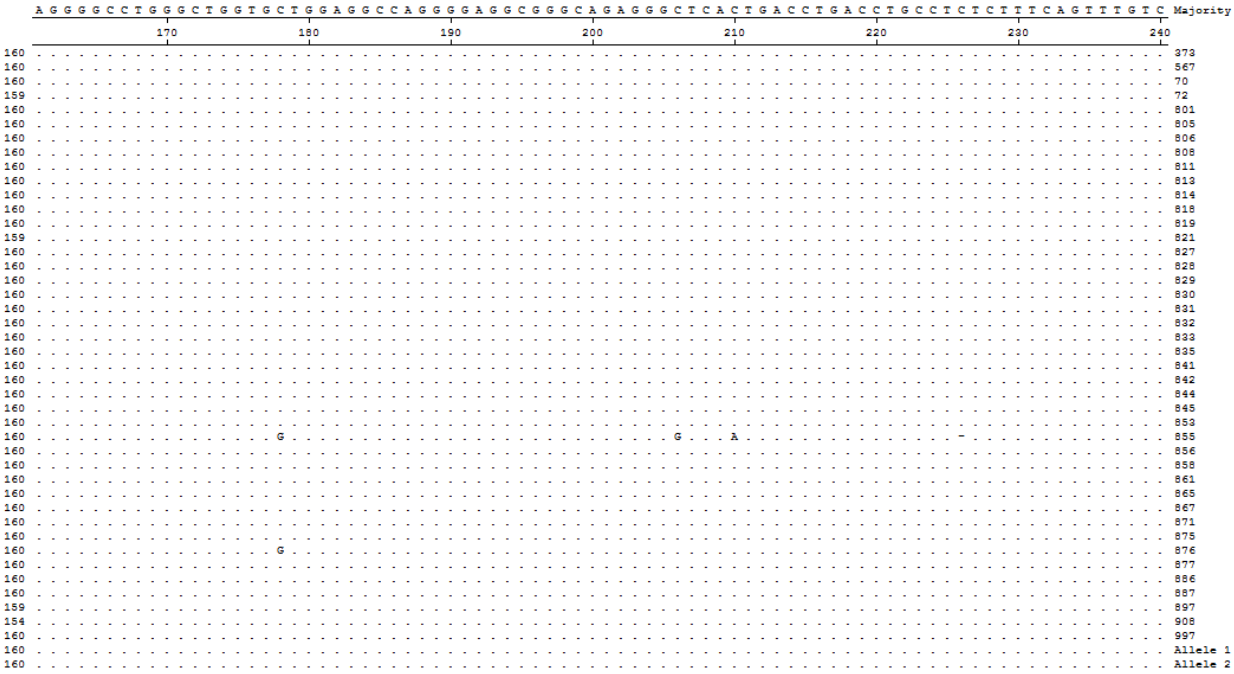

Fig.2: The nucleotide sequence alignment of IGHA with published alleles (cont...)

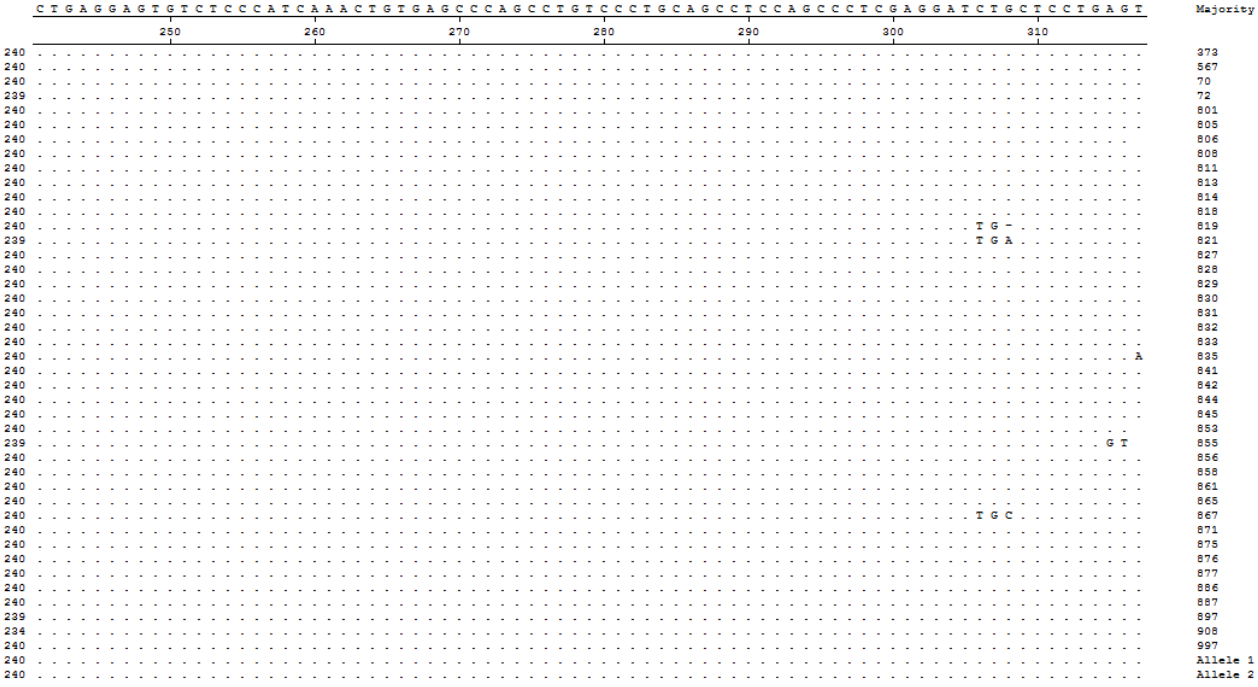

Fig.2: The nucleotide sequence alignment of IGHA with published alleles

At exon of CH2, the variations are observed at 305, 306 and 307th positions in three animals (animal nos. 821, 867 and 819), where CTG is replaced by TGA in 821, TGC in 867 and in 819 TG for CT at 305, 306th positions as well as a deletion at 307th position. The deduced amino acid for hinge region (233 to 260 of nucleotide) showed two cysteine

residues, which is responsible for flexibility of hinge region in antigen binding. The nucleotide sequence alignment of IGHA with published alleles reveals variations in 24 nucleotide positions (Fig.2) with identity of 99-100% (Fig.3). The phylogenetic analysis of IGHA alleles shows clustering of majority of animals with allele 2 (Fig.4).

Table 2: The frequency of different IGHA alleles in Kumaon hill goats

Allele	Frequency	Percent
1	6	10.3
2	52	89.7
Total	**58**	**100.0**

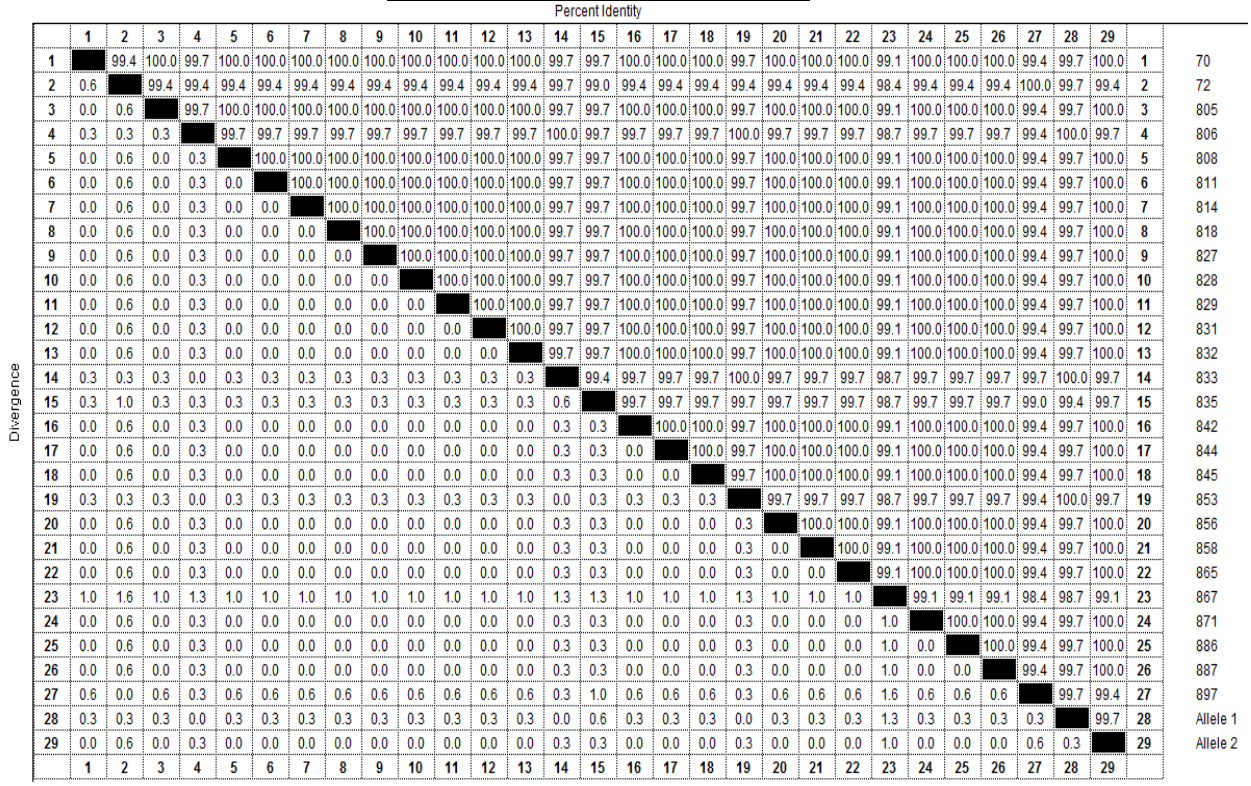

Fig. 3: Nucleotide sequence homology with published alleles

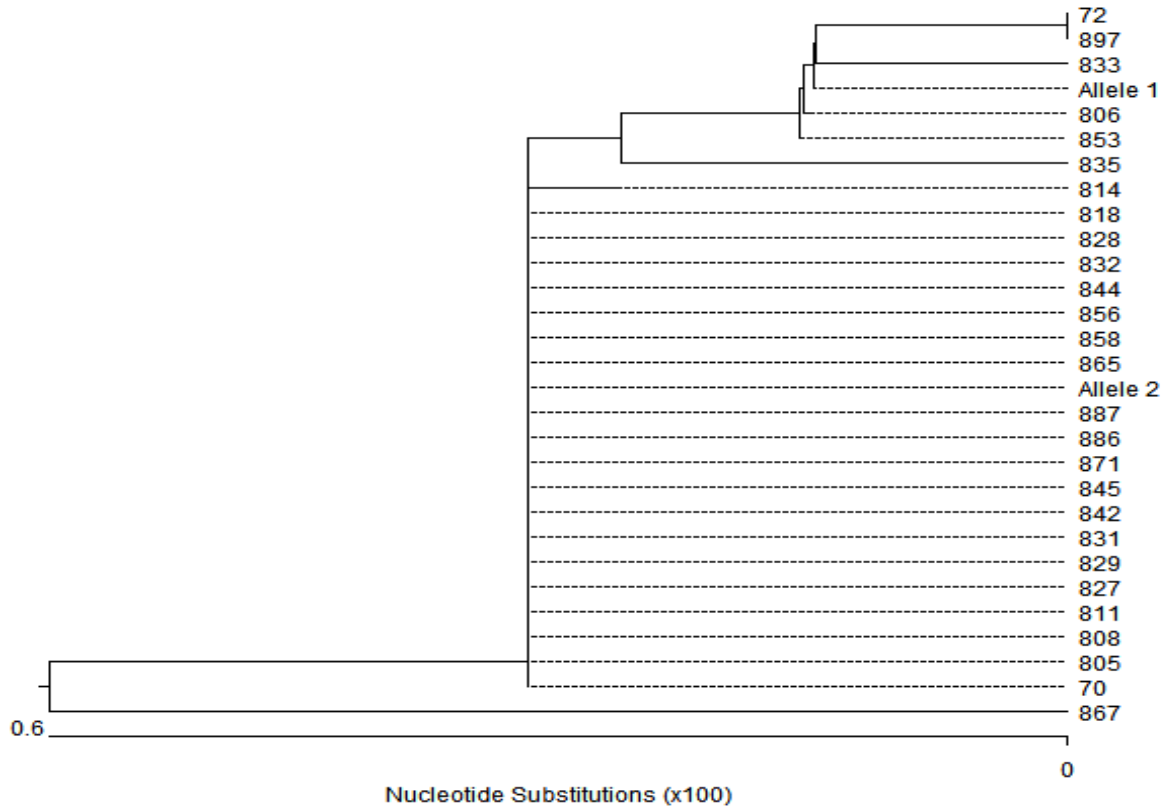

Fig. 4: The phylogenetic analysis of IGHA with the published alleles

Discussion

There was no study in hill goats on the genetic diversity and their resilience/ resistance to gastrointestinal parasites. The IGHA gene of hilly goat was successfully amplified and the amplicons size was 316bp. When the gene was analysed after sequencing, it was 316bp and consisted of truncated exon constant region (CH1), intron, hinge region and exon of constant region (CH2).

The sequencing IGHA region by Zhou et al. (2005) revealed three alleles of which, the hinge region of allele *01 codes for 2 additional amino acids when compared to that coded for by alleles *02 and *03. The allele 1 and 2 shares close homology with difference in single nucleotide variation at the carboxyl-end of the CH1 region. The allele 3 has two aminoacids shorter and has different aminoacid sequence. The sequence analysis of hilly goats revealed existence of two allele *viz*., allele 1 and allele 2 and allele 3 was absent. Though two alleles are exists, the frequency of allele 2 is more compared to allele 1.

A study has revealed allelic variation in the constant region IgA heavy alpha chain gene (IGHA) which encompasses the hinge region of the mature protein (Zhou *et al*., 2005). The IgA monomer having elongated hinge region may result in increased flexibility and their binding to wide range of epitopes. Consequently, the hinge region having additional amino

acids may also alter structure of IgA molecule such that it has less flexibility and limiting binding to parasitic epitopes. Equally, the variable hinge-region may be more or less susceptible to secreted parasite proteases (Senior and Woof, 2005). Like earlier published sequences (Accession no. AY956424–AY956426) and deduced amino acid sequences, the hinge region of IgA molecule in hilly goat (from 233 to 260 nucleotide) showed two cysteine residues, which is responsible for flexibility of hinge region for antigen binding. However, in the present study there is no difference in the hinge region of both alleles. The variation in the constant region IGHA may result in structurally and functionally different IgA molecules. Thus, the polymorphism at IGHA region has may influence the immune response to parasites and the outcome of infection. Furthermore, the variation at the IGHA locus may be acting as a proxy marker for other genes located nearby in the genome as it also linked to variation in other regions of the gene that impact on gene-expression and in this respect MHC has been implicated (Davies *et al.,* 2006). Suggesting, the location QTL associated with specific IgA activity on chromosome 20, at or near the location of the Major Histocompatability complex.

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