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THE INVESTIGATION OF THE *PRNP* GENE POLYMORPHISMS IN *OVIS ARIES ARIES* AND *CAPRA HIRCUS*

Ph.D. THESIS

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To my dear family…

Abstract

The Investigation of the *PRNP* Gene Polymorphisms in *Ovis aries aries* and *Capra hircus*

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The implementation of genetic breeding programs to eradicate transmissible spongiform encephalopathies in goats and sheeps are essential for animal breeding, animal health and welfare, together with food safety and security. Through selective breeding, the National Scrapie Plan aims to eradicate scrapie as EU countries. The aim of this research is to determine the polymorphisms related to scrapie in Chios, Awassi and hybrid sheep breeds and Damscus, Cyprus Native Hair Goat and hybrid goat breeds in the Turkish Republic of Northern Cyprus and to provide a basis for the breeding program to be made in terms of scrapie. ARQ frequency was heterozygotes for 18% and homozygotes for 24% for all sheep breeds. To yet, no investigation has been conducted in the Northern region of Cyprus. Goat breeds in Northern Cyprus are in the same situation as sheep breeds. Thus, the purpose of this study was to investigate the haplotypes related to scrapie in Northern Cyprus. R231T, a new polymorphism, was detected in the population during this analysis. The R231T polymorphism was identified in 12% of Awassi and Hybrids. To yet, no investigation has been conducted in the Northern region of Cyprus. Thus, the purpose of this study was to investigate the haplotypes related to scrapie in Northern Cyprus. This study reveals that the ARQ haplotype is 42% frequent, while the ARH haplotype is 10 percent common. Finding relevant genes for developing scrapie-resistant goats remains a difficulty. To prevent a Scrapie epidemic, stronger precautions should be taken. Goat production and native goat breeds (Cyprus Native hair Goat, Damascus) are essential to the agricultural variety of Cyprus. Therefore, it is important to comprehend the allele frequencies of Caprine Prnp gene variations for breeding efforts in Northern Cyprus. We aimed to evaluate the allele frequencies and genotype distributions of Caprine Prnp gene variants in Northern Cyprus Damascus, Cyprus Native Hair, and hybrid goats. Four distinct Northern Cyprus areas were chosen for targeted gene sequencing. Consequently, seven distinct Caprine Prnp

polymorphisms were identified: P42= (CCG>CCA), G127S (GGC>AGC), S138= (AGT>AGC), N146S (AAT>GAT), R154H (CGT>CAT), Gln172Arg (CAG>CGG), G172H (CAG>CAT), G172R (CAG>CGT), V179V (GTG>GTT), and V187 (The most prevalent genetic markers were P42 (16 percent), R154H (12 percent), and N146S (8 percent). P42=, G127S, and N146S were observed in the Damascus breed, while P42=, G127S, R154H, and N146S were observed in the Hybrid breed. The prevalence of the predisposition genotype, 146NN homozygosity, was identical to that of the Northern Cyprus Native Hair Goat. No statistically significant differences were seen between the breeds. The use of native Cypriot hair goats in animal husbandry is of crucial relevance in terms of the genetic variety of species. We believe that this breed will play a crucial role in the reduction of scrapie instances due to its genotype H154 ratio of resistance. For a comprehensive understanding of the effects of the 146D, 146S, and 154H alleles in Damascus, Cyprus Native Hair Goat, and Damascus-Saanen hybrids, larger research is required. This case-control study may provide a method for minimizing scrapie among sheeps and goats in Northern Cyprus.

Keywords: *PRNP*, sheep*,* goat*,* genotype, sequence analysis

Özet

Ovis aries ve _Capra hircus_'ta _PRNP_ Gen Polimorfizmlerinin Araştırılması

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Keçi ve koyunlarda bulaşıcı süngerimsi ensefalopatilerin ortadan kaldırılması için genetik ıslah programlarının uygulanması, hayvan ıslahı, hayvan sağlığı ve refahı ile gıda güvenliği için esastır. Seçici üreme yoluyla, Ulusal Scrapie Planı, scrapie'yi ortadan kaldırmayı amaçlar. Bu araştırmanın amacı Kuzey Kıbrıs Türk Cumhuriye'nde Sakız, Awassi ve melez koyun ırklarında ve damscus, kıbrıs yerli kıl keçi ve melez keçi ırklarında scrapieye ilşikin polimorfizimleri tespit etmek ve scrapie açısından yapılacak olan ıslah programına temel oluşturmasını sağlmakatır. ARQ sıklığı tüm ırklar için %18 heterozigot, %24 homozigot idi. Şu ana kadar Kıbrıs'ın kuzey bölgesinde herhangi bir çalışma yapılmaıştır. Keçi ırkları koyun ırkları ile aynı durumdadır. Bu nedenle bu çalışmanın amacı, Kuzey Kıbrıs'ta scrapie ile ilgili haplotiplerin araştırılmasıdır. Bu analiz sırasında popülasyonda yeni bir polimorfizm olan R231T tespit edildi. R231T polimorfizmi, İvesi ve Melezlerin %12'sinde tanımlandı. Kıbrıs'ın kuzey bölgesinde henüz bu konu hakkında herhangi bir çalışma yürütülmedi. Bu nedenle bu çalışmanın amacı, Kuzey Kıbrıs'ta scrapie ile ilgili haplotiplerin araştırılmasıdır. Bu çalışma, ARQ haplotipinin %42, ARH haplotipinin ise %10 yaygın olduğunu ortaya koymaktadır. Scrapie dirençli keçiler ıslah edebilmek için ilgili genleri bulmak oldukça zordur, bu da Scrapie salgınını önlemek için daha güçlü önlemler alınması gerektiğini göstermektedir. Keçi üretimi ve yerli keçi ırkları (Kıbrıs Yerli Kıl Keçisi, Şam) Kıbrıs'ın tarımsal çeşitliliği için esastır. Bu nedenle, Kuzey Kıbrıs'taki ıslah çalışmaları için Caprine Prnp gen varyasyonlarının alel frekanslarını anlamak önemlidir. Bu çalışmada, Kuzey Kıbrıs Damascus, Kıbrıs Yerli Kıl ve hibrit keçilerde Caprine Prnp gen varyantlarının alel frekansları ve genotip dağılımlarının değerlendirilmesi amaçlanmıştır. Hedeflenen gen dizilimi için dört ayrı Kuzey Kıbrıs bölgesi seçildi. Sonuç olarak, yedi farklı Caprine Prnp polimorfizmi tanımlandı: P42= (CCG>CCA), G127S (GGC>AGC), S138= (AGT>AGC), N146S (AAT>GAT), R154H (CGT>CAT), Gln172Arg (CAG

>CGG), G172H (CAG>CAT), G172R (CAG>CGT), V179V (GTG>GTT) ve V187 (En yaygın genetik belirteçler P42 (yüzde 16), R154H (yüzde 12) ve N146S (8 Damascus ırkında P42=, G127S ve N146S, Hibrit ırkta ise P42=, G127S, R154H ve N146S gözlenmiştir. Kuzey Kıbrıs Yerli Kıl Keçisi Irklar arasında istatistiksel olarak önemli bir farklılık görülmemiştir. Kıbrıs Yerli Kıl Keçilerinin hayvancılıkta kullanılması türlerin genetik çeşitliliği açısından çok önemlidir. Genotipi H154 direnç oranı nedeniyle scrapie vakalarının azalması 146D, 146S'nin etkilerinin kapsamlı bir şekilde anlaşılması ve Damascus, Kıbrıs Yerli Kıl Keçisi ve Damascus-Saanen hibritlerindeki 154H alelleri için daha büyük araştırmalar gereklidir. Bu vaka-kontrol çalışması, Kuzey Kıbrıs'ta koyunlar ve keçiler arasında scrapiyi en aza indirilmesi için bir yöntem sağlayabileceği tespit edilmiştir.

Anahtar Kelimeler: *PRNP*, koyun, keçi, genotip, sekans analizi

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List of Abbreviations

ACMG	American College of Medical Genetics and Genomics
AMP:	Association for Molecular Pathology
ALS:	Amyotrophic Lateral Sclerosis
BSE:	Bovine Spongiform Encephalopathy
AS:	Asymptomatic Scrapie
CJD:	Creutzfeldt – Jakob Disease
CS:	Classical Scrapie
DNA:	Deoxyribonucleic Acid
FSE:	Feline and Ungulate Spongiform Encephalopathy
GPI:	Glycosylphosphatidylinositol (GPI)
GWAS:	Genome-Wide Association Studies
IDDM:	Insulin-Dependent Diabetic Mellitus
INDEL:	Insertion/ deletion polymophisms
nsSNP:	non-synonymous SNP
PRNP:	Prion Protein Gene
PRP ^{C}:	Normal Prion Protein
PRP ^{SC}:	Scrapie Prion Protein
PrP^{Res}:	Protease-Resistant Form
SNPs:	Single Nucleotide Polymorphisms
sSNP**:**	Synonymous SNP
STR:	Short Tandem Repeats
TCNV:	Tandem Copy Number Variations
TR:	Tandem Repeats
TSE:	Transmissible spongiform encephalopathies
VNTR:	Variable Number Tandem Repeats

CHAPTER I
Introduction

Introduction

The BSE pandemic in the United Kingdom in the 1990s, as well as proof that BSE contaminated meat induces mutant CJD in humans169, piqued public interest in TSEs. The discovery that prion illnesses are protein misfolding disorders similar to Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease has sparked renewed interest in the condition. The main mechanism of protein misfolding illnesses is that misfolded proteins accelerate the refolding of normal cellular protein, which accumulates and causes brain degeneration (Hetz & Soto, 2003; Soto & Satani, 2011; Fernández-Borges et al., 2013; Prusiner, 2013; Gill & Castle, 2018). The cellular prion protein (PrP^{C}), which is encoded by the host, is misfolded into a harmful form in prion disorders (PrP^{Sc}). There is no new PrP^{Sc} creation in the absence of PrP^{C}, such as in *PRNP* knockout or PrP^{Sc} depletion. Prion illnesses differ from other protein misfolding disorders in that they might possibly spread to vulnerable persons upon natural exposure (Richt et al., 2006; Greenlee, 2019).

The *PRNP* gene is located on chromosome 20p13 in humans and chromosome 13 in sheep and goats. The *PRNP* gene encodes the prion protein (protease resistant protein, CD230 (cluster of differentiation 230, PrP^{C})) (Kretzschmar et al., 1986) which is involved in various infectious neurodegenerative spongiform encephalopathies (OMIM 176640). PrP^{C} is primarily present in mammalian neuronal cells and is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) linkage (Ambadi Thody et al., 2018). PrP^{C} occurs in many other tissues in the body, but the expression of the protein is most dominant in the nervous system (Signoret et al., 2008). The protein can present in multiple isoforms, the wild-type form showing as PrP^{C}, protease resistant forms define as PrP^{Res} and the disease-causing *PRP* illustrating as PrP^{Sc} (scrapie).

TSEs are fatal neurodegenerative disorders caused by the post-transduction change and accumulation of the pathogenic isoform of the cellular prion protein (PrP^{c}) in the central nervous system, which causes degenerative processes. Despite the fact that scrapie is an infectious illness, it is well recognized that the clinical

presentation of scrapie is significantly influenced by both the infecting agent, the prion strain, and the host's genetic vulnerability. Several polymorphisms in the coding area of the prion protein gene (*PRNP*), particularly at codons 136, 154, and 171, have a significant impact on disease susceptibility in sheep. ARQ, VRQ, ARR, AHQ, and ARH are the six major alleles encoded by these three nucleotide triplets (Vaccari et al., 2009; Bordin et al., 2020) .

Genetic variation within the *PRNP* gene affects the susceptibility of infectious spongiform encephalopathies. These deadly neurodegenerative diseases are caused by an infectious form of misfolded prion protein. Therefore, altered protein might affect humans as well as sheep, goats, cattle, mule deer, and elks (Bernardi & Bruni, 2019).

The *PRNP* gene of goats has been well studied, revealing a considerable number of polymorphisms. Twenty-five amino acid replacements have been determined in common and domestic European goat breeds (Vaccari et al., 2009). The investigation of the PRNP gene of goats found a significant number of polymorphisms in this species as well. Particularly, 25 amino acid substitutions have been identified in domestic and popular European goat breeds. In non-European races and populations, these and a few unique PRNP polymorphisms have been identified: (China) W102G (displaying the wild-type amino acid, codon, and variant), S240P, G127S, Q222K, H143R, R211G, N146S, T219I, R154H, R211Q, and I218L (exhibiting the variant amino acid and codon) (Zhou et al., 2008); (Japan) *I142M, G127S, H143R, S240P, N146S, R211Q*; (Pakistan) *S240P* (Babar et al., 2009); and (USA) *I142M, G127S, H143R, N146S, Q222K, R154H, R211Q, S240P (White et al., 2008,* Meydan et al., 2017). In essence, there are numerous amino acid polymorphisms emerging from relatively few countries. Nevertheless, 7 out of the 29 reported amino acid alterations appear to have a global distribution (by haplotype gradation, from most prevalent: *R143, P240, S127, K222, H154, S146, Q211, M142* and a minimum five of these have been offered to be associated with TSE sensitivity (Vaccari et al., 2009).

Genetic Variations

The extent to which a species' ability to withstand both short and long-term illness conditions is related to the great level of genetic variety exhibited within and between populations has long been debated (Schierenbeck, 2017). The difference in DNA between individuals or between groups is referred to as genetic variation

Genetic diversity may come from a variety of places, including mutation and genetic recombination. While mutations are the primary cause of genetic diversity, other factors like as sexual reproduction and genetic drift also play a role (*Rethinking Evolution: The Revolution That's Hiding In Plain Sight by Gene Levinson | 9781786347268 | Hardcover | Barnes & Noble®*, n.d.). Variation in genetics can be detected on a variety of levels. Identifying genetic variation requires observations of phenotypic variation in either quantitative traits (traits that vary continuously and are coded for by many genes) or discrete traits (traits that fit into defined categories and are coded for by one or a few genes). Polymorphisms are less common in vertebrates than in insects and plants, where fifty percent of enzyme-coding genes are polymorphic (*Genetic Variation Scientific Journals | Peer Reviewed Articles*, n.d.). If differences in the order of nucleotides in the DNA sequence result in differences in the order of amino acids in proteins coded by that DNA sequence, and if the resulting differences in amino acid sequence influence the shape, and thus the function, of the enzyme, then genetic variation will result in phenotypic variation (Pavlopoulos et al., 2013).

Types of variations

When so much of genetics is concerned with the study of variations, it is critical to understand the many forms of variation observed in populations. A relevant distinction is made between discontinuous and continuous variation. Both are influenced by allelic variation. Natural populations exhibit both discontinuous and continuous variation. Although discontinuous variation is a simpler type of variation and easier to study, discontinuous variation has been the focus of most genetics research during the last century. A feature is found in a population in two or more different and independent forms termed phenotypes in discontinuous variation. Alternative phenotypes are frequently discovered to be encoded by alleles of a single gene (*Genetic Variation - An Introduction to Genetic Analysis - NCBI Bookshelf*, n.d.)**.**

Single Nucleotide Polymorphisms (SNPs)

A single nucleotide polymorphism (SNP) is a single difference in the nucleotide sequence of DNA that can impact or not impact the phenotypic characteristics of an organism. That it is a single base change in genomic DNA with sequence alternatives in normal individuals and the least common allele having a frequency of at least 1% (Bin et al., 2014). Therefore, the term SNP is frequently used in a broader sense to

refer to any single base mutation that is identified and may be linked to a disease. The word SNP will be used in this thesis in its broadest sense. More than 99 percent of human DNA is shared, while SNPs account for 90% of the 1% that varies (Fairweather-Tait et al., 2007). SNPs have an impact on how a person reacts to illness, medicines, and environmental circumstances. Every 300 bases in human DNA, on average, there is a single nucleotide polymorphism (SNP). This suggests that there are around 10 million SNPs throughout the entire genome (3 billion bases) (Burton et al., 2007; Ma & Lu, 2011; Bresciani et al., 2013; Nelson et al., 2004). In the coding areas of the genes, there are about 60,000 SNPs (Sachidanandam et al., 2001, Bin et al., 2014).

Types of SNPs: SNPs are classified according to where they are found in the genome: in gene coding sections, non-coding portions of genes, or intergenic regions. The bulk of SNPs are found in non-coding areas of the genome (Syvänen, 2001). SNPs in the coding region, on the other hand, are important because they can change a variety of biological and molecular activities, including stability, expression level and protein function (Casadio et al., 2011, Chasman & Adams, 2001, Bin et al., 2014). Approximately 3–5% of the estimated 10 million SNPs are found in the coding area; half of them result in amino acid alterations, known as non-synonymous SNPs (nsSNPs). SNPs in coding areas are categorized based on their impact. A synonymous SNP (sSNP) is one that does not modify a protein's amino acid sequence, whereas a non-synonymous SNP (nsSNP) does. It has been estimated that up to 20% of nsSNPs may cause protein damage A missense nsSNP causes a different amino acid to be produced, whereas a nonsense nsSNP causes a premature stop codon to be produced. Missense nsSNPs can be conservative or non-conservative in nature. When you make a non-conservative modification, you get a new residue with drastically altered physicochemical characteristics (Bin et al., 2014).

Tandem repeat polymorphisms

Tandem repeats (TR) are patterns of similar subsequence's that are physically next to each other in DNA sequences. TR is abundant in the human genome, and its research is crucial for a variety of applications in forensics, medical genetics, and population research. TR is classified into microsatellites when the number of bases in the repeating unit is between 1 and 6 bps (for some authors from 1 to 10 bps), minisatellites when the number of bases in the repeating unit is between 7 and 50 bps

(for some authors the range stretches from 10 to 100 bps), and satellites when the number of bases in the repeating unit is greater than 50 bps, according to a standard classification based on the number of bases in the repeating unit (Genovese et al., 2018). Tandem Copy Number Variations (TCNV) are TRs with repetition units ranging from 1 to 10 Kb on a string (He et al., 2011). When the emphasis is placed on their highly polymorphic character, microsatellites are also referred to as Short Tandem Repeats (STR) in the scientific literature, whereas minisatellites are referred to as Variable Number Tandem Repeats (VNTR) in the scientific literature (Gelfand et al., 2014).

The biological factors that separate these groups, particularly VNTR vs. STR, are many. Variability in the number of repeating units of VNTR and STR loci in a population is caused by different molecular causes. The majority of repeat number variability in STR is caused by strand slippage during DNA polymerase replication (Fan & Chu, 2007)(Mirkin, 2007). Variation in the amount of repeat units in VNTR is largely caused by uneven sister chromatid exchange events (Genovese et al., 2018). More than 30 illnesses, predominantly neurodegenerative and neuromuscular illnesses, are caused by TR expansions, including Huntington disease (HD), Kennedy disease (SBMA), and several types of Spinocerebral Ataxias (SCA) (Orr & Zoghbi, 2007).

Microsatellites (short tandem repeat)

Short tandem repeats (STRs), also known as microsatellites or simple sequence repeats, are short tandemly repeated DNA sequences with a repeating unit of 1-6 bp that may create series of up to 100 nucleotides in length (nt) (Fan & Chu, 2007). Prokaryotes and eukaryotes, including humans, have a lot of STRs. They appear to be distributed quite uniformly across the human genome, accounting for around 3% of the total genome. However, their distribution throughout chromosomes is not completely uniform; sub telomeric areas appear less often (Koreth et al., 1996). The most of STRs have been in the noncoding regions, approximately 8% locate in the coding regions (Fan & Chu, 2007).

Repetitive DNA makes up around two-thirds of the human genome (de Koning et al., 2011). Microsatellites are the simplest of these repetitions in terms of size, complexity, and quantity in the genome. The human genome contains around 600,000 distinct microsatellites, each consisting of a small motif (1–6 base pairs) repeated in tandem to form an array (Ellegren, 2004, Borštnik & Pumpernik, 2002,

Li et al., 2004). Microsatellites have been used in forensic and kinship analyses for decades, despite their simplicity. Because of their high mutation rate, they essentially act as genetic fingerprints. Microsatellites also play an important part in disorders such as fragile X syndrome, spinocerebellar ataxia, myotonic dystrophy, Friedrich ataxia, and Huntington's disease (Murmann et al., 2018; Everett, 2010; Orr & Zoghbi, 2007).

Microsatellites have recently received a lot of attention because of their involvement in complicated diseases and subtler effects on gene expression (Sawaya et al., 2012). Through inducing Z-DNA and H-DNA folding, modifying nucleosome position, and modifying the spacing of DNA binding sites, changes in the length of repeat arrays could influence gene expression (Sawaya et al., 2012;Vinces et al., 2009). Actually, a recent genome-wide investigation of short tandem repeats (STRs) discovered 2,060 that influence near-by gene expression (eSTRs) and calculated that STRs increase by up to 15% of the cis heritability among all types of genetic variations (Ellegren, 2004; Gymrek et al., 2015; Bacolla & Wells, 2009; Sawaya et al., 2012).

Minisatellites

Since around 1970, our knowledge of DNA has changed dramatically in two areas: DNA stability and the organization of genetic material in life forms. Previously, DNA was thought to be a very stable object that only underwent minor changes due to infrequent mutational occurrences. This idea was backed up by experimental data from the 1950s, which proved that DNA replication occurs with a very low mistake rate. The "Central Dogma," which states that the flow of information in cells travels from DNA to RNA and then from RNA to proteins, exemplifies the genetic system's stability and accuracy (Ramel, 1997).

A minisatellite is a fragment of repetitive DNA in which certain DNA patterns (between 10 and 60 base pairs in length) are repeated 5 to 50 times. Minisatellites are found in over 1,000 places across the human genome and are known for their high mutation rate and population diversity (Tawn et al., 2011). Minisatellites are prevalent in chromosome centromeres and telomeres, the latter of which protects the chromosomes from harm. The term "satellite" comes from the discovery that spinning of genomic DNA in a test tube separates a conspicuous layer of bulk DNA from a layer of repeated DNA called "satellite." Minisatellites are short DNA sequences that do not encode proteins but are found hundreds of times across the

genome, with several copies standing adjacent to each other (*Comparison and Correlation of Simple Sequence Repeats Distribution in Genomes of Brucella Species*, n.d.).

Insertion/ deletion polymorphisms (INDEL)

Continuous progress in the way that genetic similarity or difference between genomes is measured has followed the progress, or perhaps better put, progress in population and evolutionary genetic studies. In the long run, the genetic marker technique has shifted from phenotypes to genotypes through immunological parameters and proteins (Schlotteröer et al., 1991; Ellegren, 1992; Väli et al., 2008). New genetic markers in the research of natural populations however have followed methodological improvements accomplished in the genetic analysis of model organisms (Schlötterer, 2004). An insertion/deletion polymorphism mostly abbreviated "indel," is a kind of genetic variation in that a specific nucleotide sequence is present (insertion) or absent (deletion). Indels, though not as prevalent as SNPs, are found throughout the genome. Indels make up 3 million of the 15 million genetic variations that have been identified. A frameshift mutation occurs when an indel in a gene's coding sequence is not a multiple of three nucleotides. Shifting the reading frame and the DNA transcript sequence may now code for a whole new set of amino acids or result in a premature stop codon, changing the structure and function of the protein. Indel variations with multiples of three nucleotides result in a protein with additional amino acids (insertion) or amino acid loss (deletion), but no effect on the other amino acids. (Boltz et al., 2013).

Gross chromosomal aberrations

Chromosomal aberrances are basic categorized into two groups: structural and numerical alterations (Grade et al., 2015). Gross structural rearranging involves translocation, chromosomal changes including deletion, duplication, gene amplification, and inversion, while numerical abnormalities front to the abnormal number of a complete chromosome or whole chromosome set, resulted in aneuploidy and polyploidy, respectively (Aygun, 2017).

Structural chromosomal abnormalities: Structural abnormalities occur when a portion of a chromosome is missing, duplicated, transferred to another chromosome, or flipped upside down.

Chromosomal anomalies can occur by chance during the formation of the egg or sperm, or during the early stages of the fetus' development. Genetic mistakes may be

influenced by the mother's age as well as certain environmental circumstances. Some, but not all, forms of chromosomal abnormalities can be detected by prenatal screening and testing of the fetus's chromosomes.

Breakage and improper reconnection of chromosomal segments causes structural chromosomal defects. Disease is caused by a variety of structural chromosomal abnormalities. Balanced structural rearrangements are those in which the entire chromosomal set is still intact, albeit reorganized, whereas unbalanced structural rearrangements are those in which extra or missing information is present. Deletions, duplications, and insertions of chromosomal segments are examples of unbalanced rearrangements. When a chromosome breaks twice and the fragmented ends combine to form a circular chromosome, ring chromosomes form. When one arm of a chromosome is absent, the remaining arm multiplies, becoming an isochromosome (Aygun, 2017).

The role of genetic variations in diseases

Livestock are fundamental to the livelihoods of livestock keepers, consumers, marketers, and employees across the world. Animal illnesses can have a substantial influence on animal productivity and output, human health (diseases transmissible from animals to humans), and, as a result, the entire economic development process.

In the global economy, breeding has mostly concentrated on product qualities like as milk, meat, eggs, and fiber, with medicines serving as the primary disease management method. The outcome is a significant loss in livestock populations' genetic potential to resistance or tolerate infection, while parasites - worms, bacteria, and viruses – increase their genetic infectivity (Jovanović et al., 2009).

Efforts to manage illness in livestock continue to make substantial progress, for major gains in both performance and welfare. Improved understanding of disease biology and epidemiology, as well as the discovery of medicines like antibiotics and anthelmintics, have all contributed to better disease control (Berry et al., 2011).

When it comes to creating breeding programs in veterinary medicine, it's critical to understand the difference between resistance and tolerance. The capacity of a host to fight infection is referred to as resistance, whereas tolerance refers to a state in which the host is infected by the pathogen but exhibits very minimal side effects. If the intention is to keep infection from spreading to other populations, such as with zoonoses, disease resistance is significantly more beneficial than tolerance. Animal resistance to infections caused by pathogens of different etiologies can be determined

genetically at three levels: species, breed, and individual animal genetic diversity. When evaluating the importance of resistance/tolerance at the breed level, it's important to consider the intrinsic evolutionary advantage of breeds that are well-adapted to their environments (Jovanović et al., 2009).

Conversely, increase health in animal breeding and genetics, both of which are essential for animal disease control, have been made. The relationship between the animal's genetic composition and the specific environment it was exposed to is the result of observable animal performance, which is of great veterinary interest. As a result, improving genetics has the potential to complement present animal disease management methods. Genetic selection is helpful for improving animal health because genetic gain is cumulative and permanent, since genes introduced into a population can survive for many generations. Trying to unravel the genetic architecture of health and disease resistance not only promotes the creation of understanding on breeding potential for enhanced health status, but it also provides information for scientific research in animals and humans, especially vaccine development (Berry et al., 2011).

Genetic epidemiological studies

Genetic epidemiology is a new medical field that studies how genetic variables interact with the environment in the setting of disease in communities. The causes of hereditary illness, as well as its distribution and management, are all areas of research.

Epidemiology is not a science in and of itself. In recent years, the addition of genetic to the word epidemiology has connoted the effect of genetic variables on disease incidence or other qualities that describe individuals in a community, whether those individuals are humans or other individuals in a population; most typically people. Importantly, because genetic epidemiology is concerned with people in their environment, what one sees in genetic epidemiology is not just the genetic traits of individuals within a community, but also the environmental impacts that those groups face in their surroundings (*Genetic Epidemiology*, n.d., 2022).

Traditional Epidemiologic Studies: While cohort, cross-sectional, and case-control studies may all be used to investigate genetic variables in illness, the case-control technique is especially well adapted to genetic epidemiology. There are several causes for this: 1) Unlike biological exposure markers (e.g., occupational, nutritional), genetic markers are persistent indications of host vulnerability; 2) case-control studies may be used to "fish" for the impacts of many genes, as well as other risk factors, and to seek for gene-environment interaction. 3) Case-control studies are appropriate for a variety of rare illness outcomes, including birth abnormalities and particular malignancies.

Studies that Assess Unmeasured Genetic Factors: Inbreeding and racial/ethnic mixing studies are discussed in this section.

Inbreeding Studies: Inbreeding has the overall effect of increasing the chance of homozygozity at each autosomal locus, and consequently the prevalence of detrimental recessive genetic characteristics in the population. Autosomal recessive illnesses are projected to become more common as a result of inbreeding (such as cystic fibrosis and phenylketonuria). Even if a recessive genetic component for the disease cannot be directly detected, inbreeding studies can be utilized to evaluate a recessive genetic component for diseases with unclear origin. Despite the fact that inbreeding is on the decline globally, certain populations still have a high rate of consanguineous marriages (20% – 50%) and are thus still ideal for analyzing inbreeding consequences (*Genetic Epidemiology*, n.d.).

The individual's inbreeding coefficient is the "exposure" variable of interest in inbreeding investigations. The inbreeding coefficient is the likelihood that a person contains two alleles that are identical by ancestry at each autosomal locus. Inbreeding coefficients for frequent patterns are known (for example, 1/16 for first cousin marriages, 1/32 for first cousins once removed marriages, and 1/64 for second cousin marriages). In general, route techniques or iterative calculation may be used to derive inbreeding coefficients from long pedigrees. Researchers must use suitable control groups when planning inbreeding case-control studies, because inbreeding is linked to a variety of demographic, cultural, religious, and geographic characteristics that may or may not be connected to the illness of interest (and thus are potential confounders). While it is not always essential to match for such demographic characteristics, proper stratification and adjustment is generally required when examining inbreeding effects in case-control studies.

Admixture Studies: Admixture studies are a valuable epidemiologic method for evaluating the relative impact of genetic variables when the prevalence of a disease differs by racial/ethnic group. If the frequency of particular genetic features differ between two groups, intergroup mating will raise the possibility of heterozygozity in the progeny, resulting in either disruption of previously adapted genotypes or beneficial impacts. Admixture experiments can be helpful in identifying key genetic variables. It has been suggested, for example, that genetic mingling may have contributed to the higher prevalence of insulin-dependent diabetic mellitus (IDDM) among U.S. blacks than among African blacks (*Genetic Epidemiology | Books | CDC*, n.d.,).

Genome-Wide Association Studies (GWAS) Studies

A genome-wide association study is a method of searching for genetic variants linked to a disease by rapidly screening markers throughout the whole sets of DNA, or genomes, of many living creatures. Researchers can utilize the knowledge to build better ways to diagnose, cure, and prevent disease whenever new genetic connections are discovered. These kinds of research are especially beneficial for identifying genetic variants that contribute to prevalent, complicated diseases including asthma, cancer, diabetes, heart disease, and mental disorders.

Researchers now have a set of research methods to discover the genetic contributions to prevalent illnesses, thanks to the completion of the Human Genome Project in 2003 and the International HapMap Project in 2005. Computerized databases containing the reference human genome sequence, a map of human genetic diversity, and a set of innovative technologies capable of swiftly and reliably analyzing whole-genome samples for genetic variants that contribute to the development of illness are among the tools.

The influence of genome-wide association research on medical treatment might be significant. Such research is establishing the basis for the development of personalized medicine, in which the present one-size-fits-all approach to medical care will be replaced by more tailored approaches. Health practitioners will be able to utilize genome-wide scans and other new technologies to give patients with customized information about their chances of acquiring specific diseases in the future, after the cost and efficiency of such scans and other innovative technologies has improved. Health practitioners will be able to adapt preventive strategies to each person's genetic composition using this knowledge. Furthermore, if a patient

becomes unwell, the information may be utilized to choose the therapies that are most likely to be successful and have the fewest side effects in that patient (*Genome-Wide Association Studies Fact Sheet*, n.d.). Various substantial connections between genetic polymorphisms and susceptibility to major infectious disease phenotypes, such as HIV-1, hepatitis B and C viruses, dengue, malaria, TB, leprosy, meningococcal illness, and prion disease, have been discovered in genome-wide association studies (Chapman & Hill, 2012).

American College of Medical Genetics and Genomics (ACMG)

Clinical geneticists, clinical laboratory geneticists, and genetic counsellors are all represented by the ACMG, which is the only nationally recognized multidisciplinary professional membership organization that promotes the interests of the complete medical genetics team (ACMG, 2022.). The ACMG–AMP (American College of Medical Genetics and Genomics–Association for Molecular Pathology) recommendations for the interpretation of sequence variations, published in 2015, were a major step in establishing a common framework for variant categorization (Richards et al., 2015).

Variant Classifications

Clinical molecular genetic testing relies on the categorization of variants. Variant classifications must be evidence-based, objective, and systematic for genetic testing to be reliable and useful (Rehm et al., 2015;Garcia et al., 2016). Pathogenicity assessments must be repeatable and free of personal and professional biases that might exist in research labs, diagnostic laboratory investigative settings, and doctors' pressing need to establish a diagnosis (Nykamp et al., 2017).

Genetic Susceptibility

Increased possibility or chance of acquiring a certain illness owing to one or more gene mutations and/or a family history of the disease. Genetic predisposition is another term for it (*Definition of Genetic Susceptibility - NCI Dictionary of Genetics Terms - National Cancer Institute*, n.d.). Early investigations of infectious illness susceptibility included genome-wide linkage analysis and candidate-gene techniques, but only found a few significantly linked loci that were independently replicated. Linkage approaches have been employed successfully in monogenic disease studies and were subsequently applied in attempts to define the susceptibility loci underlying common diseases. The research of afflicted sibling pairs was the most extensively

used strategy, and it was successful in discovering loci connected to various infectious illnesses, such as leprosy (Cooke & Hill, 2001; Misch et al., 2010). The difficulties in enrolling multiple multicase families in which two siblings are afflicted by infectious illness, as well as a lack of research power, restrict linkage analysis (Burgner et al., 2006; Chapman & Hill, 2012; Cooke & Hill, 2001).

Prion diseases

The PRNP gene is located on chromosome 20p13 in humans and chromosome 13 on sheep and goats. The PRNP gene encodes the prion protein (protease-resistant-protein, CD230 (cluster of differentiation 230, PrPC) (Kretzschmar et al., 1986) which is involved in various infectious neurodegenerative spongiform encephalopathies (OMIM 176640). PrPC is primarily present in mammalian neuronal cells and is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) linkage (Ambadi Thody et al., 2018). PrPC occurs in many other tissues in the body, but the expression of the protein is most dominant in the nervous system (Zomosa-Signoret et al., 2008). The protein can present in multiple isoforms, the wild-type form showing asPrPC, protease-resistant forms define as PrPRes and the disease-causing PRP illustrating as PrPSc (scrapie). Scrapie, the first and most ancient animal Transmissible Spongiform Encephalopathy, has been given the superscript (Sc) (TSE). To distinguish between normal and pathogenic (disease-causing) isoforms, several writers employ superscripts other than (Sc) (Imran & Mahmood, 2011; Schmitz et al., 2017).

Genetic variation within the PRNP gene affects the susceptibility of infectious spongiform encephalopathies. These diseases are a family of fatal neurodegenerative diseases that caused by an infectious and results misfolded isoform of prion protein. Therefore, altered protein might affect humans as well as sheep, goats, cattle, mule deer, and elks (Bernardi & Bruni, 2019). Direct contact and particularly ingestion of infected material or tissues appear to be the primary mode of transmission for most acquired prion diseases (Miller & Walter, 2019). PrPSc neurodegenerative by species diseases are shown in Table 1.

Table 1.

The misfolded version PrP^{Sc} is incorporated with a variety of cognitive deformity and neurodegenerative diseases

Animal prion diseases

Disease	**Host**	**Etiology**	**Year of Description**	**References**
Scrapie	Sheep, Goats	Unknown source of prion infection	Mid 18th century	(Aguzzi. A, 2006, Imran & Mahmood, 2011a, 2011b)
Transmissible mink encephalopathy (TME)	Mink	Infection with Prions of either sheep or cattle origin	1947	(Sigurdson & Miller, 2003, Imran & Mahmood, 2011a, 2011b)
Chronic wasting disease (CWD)	Cervids	Infection with Prions of unknown origin	1967	(Williams ES & Young S, 1980, Sigurdson & Miller,

				2003, Imran & Mahmood, 2011a, 2011b)
Bovine spongiform encephalopathy (BSE)	Cattle	Infection with Prions of unknown origin	1986	(Wells GA et al., 1987)
Exotic ungulate spongiform encephalopathy (EUE)	Nyala, Kudu	Infection with Prions of BSE origin	1986	(Kirkwood JK & Cunnigham AA, 1994)
Feline spongiform encephalopathy (FSE)	Cats	Infection with Prions of BSE origin	1990	**(**Imran & Mahmood, 2011a, 2011b)
Feline spongiform encephalopathy (NHP)	Lemurs	Infection with Prions of BSE origin	1996	(Bons N et al., 1999)
Human prion diseases				

Disease	Host	Etiology	Year of Description	References
Kuru	Human	Ritualistic Cannibalism or "Transumption"	1957	**(**Imran & Mahmood, 2011a, 2011b)
Sporadic CJD (sCJD)	Human	Spontaneous $PrP^{C} \rightarrow PrP^{Sc}$ conversion or somatic mutation	1920	**(**Imran & Mahmood, 2011a, 2011b)
Familial/genetic CJD (f/gCJD)	Human	Mutations in *PRNP*	1924	**(**Imran & Mahmood, 2011a, 2011b)
Gertsmann-Straussler-Scheinker syndrome (GSS)	Human	Mutations in *PRNP*	1936	**(**Imran & Mahmood, 2011a, 2011b)

Iatrogenic CJD (iCJD)	Human	Infection with Prions of human origin by cadaveric corneal grafts, hGH or dura mater	1974	**(**Imran & Mahmood, 2011a, 2011b)
FFI	Human	*PRNP* haplotype 178N-129M	1986	**(**Imran & Mahmood, 2011a, 2011b)
vCJD	Human	Infection with Prions of BSE origin	1996	**(**Imran & Mahmood, 2011a, 2011b)
sFI	Human	Somatic PrP^{C} PrP^{Sc} conversion or spontaneous PrP^{C} PrP^{Sc} conversion	1999	**(**Imran & Mahmood, 2011a, 2011b)
VPSPr	Human	Spontaneous PrP^{C}→PrP^{Sc} conversion or somatic mutation	2008	**(**Imran & Mahmood, 2011a, 2011b)

Prion Diseases in Animal

Scrapie

Scrapie, the most common kind of transmissible spongiform encephalopathy (TSE), is a prion disease that affects sheep, goats, and mouflons (Ovis musimon) (JL & SH, 1992). In the 16th century, scrapie was first described in Europe (England 1732 and Germany 1759; Gaiger, 1924; Mathiason, 2017). This is most likely why the illness is known by so many different names, including:shakings/shakers, cuddie trot, rida (ataxia or tremor), rickets, rubbers, goggles (to stare or squint), la tremblante (trembling), traberkrankheit (trotting disease), and prurigo lumbar (itchy back) (*Gap Junctions - E.L. Hertzberg - Google Books*, n.d.). Scrapie is the first prion disease that has been demonstrated to be both contagious and transmissible in natural settings (Mathiason, 2017).

Scrapie became a financial problem for sheep farmers in the early twentieth century (between 1920 and 1958). Scrapie has impacted almost all sheep breeds, however it is more frequent in some than others. Scrapie was introduced into many nations as a result of international commerce of infected Suffolk sheep, resulting in endemic illnesses in flocks all over the World (Kittelberger et al., 2010)(de Andrade et al., 2015).

Classical and atypical types of natural scrapie in sheep and goats are differentiated by neuropathology and PrPSc glycosylation patterns on western blots (S. L. Benestad et al., 2003;Tranulis et al., 2011). The first case of atypical scrapie was reported in 1998, and it has since been found throughout Europe, primarily via diligent observation of asymptomatic sheep (Polak et al., 2010; Nentwig et al., 2007; De Bosschere et al., n.d.). The first case of atypical scrapie in sheep was discovered in 1972 (Chong et al., 2015). Older sheep with *PRNP* genotypes that are resistant to classical scrapie (ARR/ARR) are more likely to develop atypical scrapie (G. Lühken et al., 2004; Gesine Lühken et al., 2007; Sylvie L. Benestad et al., 2008). Both kinds of scrapie (classical and atypical) have been seen in the same naturally diseased animal, raising issues about where atypical scrapie came from. According to experimental inoculation investigations, the phenotypic of atypical scrapie can transform into that of a second strain during transit in sheep in some cases (Chong et

al., 2015; Langeveld et al., 2014; Mazza et al., 2010). As a result, spontaneous TSE infections in ruminants are more likely to involve a combination of strains rather than a single strain (Chong et al., 2015; Mathiason, 2017).

Classical Scrapie: Classical Scrapie in sheep and goats is a prion disease that was originally documented approximately 300 years ago in the United Kingdom and other Western European countries. Scrapie has been reported all over the world since then, with the notable exception of Australia and New Zealand. Scrapie can spread quickly within a flock. It results in annual economic losses of up to $20 million in the United States due to decreased productivity, export losses, and increased carcass disposal costs (Greenlee, 2019). Prion disorders, often known as TSEs, are a series of deadly neurodegenerative illnesses that affect both animals and humans. Several illnesses with varied origins and epidemiologies (sporadic, hereditary, or acquired) are all molecularly based on the conversion of the host-encoded cellular prion protein (PrP^{C}) into a disease-associated isoform (PrP^{Sc}), which is thought to be the major component of the prion agent. PrPSc forms by attracting PrPC to aggregates that turn PrP^{C} into PrP^{Sc}. TSEs have been found in animals that are part of the human food chain, including small and wild ruminants, cattle, and even camels. Indeed, zoonotic transmission is feasible, at least in the case of classical bovine spongiform encephalopathy (BSE-C), which causes variant Creutzfeldt-Jakob disease (vCJD) in humans and was responsible for one of the most serious food safety crises ever observed (Marín-Moreno et al., 2021).

Atypical Scrapie: An atypical form of scrapie caused by the Nor98 strain was first found in Norway in 1998; nevertheless, it took until 2003 for its official scientific description, which was afterward described throughout Europe and, finally, globally. The clinical, pathological, biochemical, and epidemiological characteristics of classical and atypical scrapie differ significantly. PrPSc deposits and lesions are mostly seen in the cerebellum in atypical scrapie, rather than the obex as in classic scrapie. Although infectivity has been proven in the lymphoreticular system (LRS), nerves, and muscles, PrPSc has not been found in peripheral tissues. Classic scrapie has an unknown origin. Scrapie was a contagious and infectious disease in sheep, according to many accounts dating from the 18th to the early 19th century. The cause of atypical scrapie is similarly unknown. It is thought to be a sporadic condition with no evidence of infection at this time (Acín et al., 2021). Atypical scrapie is a non-contagious form of the disease, according to pathogenesis. Although PrP^{Sc} is not

identified in peripheral lymphoid tissue, infectivity has been demonstrated in lymphoid tissue, nerves, and muscles using a bioassay with transgenic mice. In atypical scrapie, the distribution is not primarily focused in the medulla oblongata, but the cerebellum contains the highest concentration of prion proteins (Cassmann et al., 2021).

Polymorphisms in the prion protein gene are linked to sheep's susceptibility to CS (PRNP). Codons 136, 154, and 171 have polymorphisms linked to CS susceptibility or resistance. Sheep with the VRQ and ARQ haplotypes are sensitive to CS, although the amino acid polymorphisms A136, R154, and R171 are linked to relative resistance to the disease. Natural cases of AS exist in sheep with the AHQ, ARQ, and ARR haplotypes, and a polymorphism in the PRNP gene that replaces phenylalanine (F) at codon 141 enhances the risk of AS (Cassmann et al., 2021).

Goats bearing the allele with three octapeptide repeats and the W102G mutation have a reduced vulnerability to scrapie, according to research. According to studies, the R143 and H154 polymorphisms in particular Greek goat breeds may provide some protection against natural scrapie. Similarly, R154H and R211Q variants were linked to a low sensitivity to scrapie, despite the R154H polymorphism being a risk factor for atypical scrapie. (Acín et al., 2021).

Transmissible mink encephalopathy

TME is a relatively rare illness in ranch-raised mink, but it may have terrible repercussions, eradicating an entire herd of mature breeding animals in certain cases. An external source of infection causes the illness, and (*Ch03*, n.d.)mink are exposed to it through contaminated feed. Mink ranchers frequently use untreated butcher waste and dead animals in their diet.

Even though it has not been able to document the feeding of sheep material in all outbreaks, a direct relationship between scrapie and TME is plausible since sheep (and maybe goats) are the only known animal reservoirs of scrapie-like agents in nature. BSE can be assumed to be the same way. TME does, in fact, serve as a model for the formation of BSE, but the conditions of infection differ. TME is related to infrequent, geographically isolated, comparably high levels of infection in untreated slaughterhouse waste, whereas BSE is linked to widespread, long-term exposure to a very low level of infection in processed animal waste (at least in the United Kingdom).

TME is a "dead-end" disease, which means there are no natural routes for transmission from mink to mink until cannibalism occurs. It differs substantially from scrapie in this aspect, and it can be used as a model for a different future route for the BSE pandemic (Naslavsky et al., 1997).

Chronic wasting disease

Deer, elk, reindeer, sika deer, and moose are all affected by chronic wasting disease (CWD), which is a prion illness. It has been discovered in Canada, the United States, Norway, and South Korea, among other places in North America. An infected animal's symptoms, which include severe weight loss (wasting), tripping, listlessness, and other neurologic abnormalities, might take up to a year to manifest. CWD may harm animals of all ages, and some infected animals may die before the illness manifests. Animals die from CWD, and there are no therapies or vaccinations available ("Annual Report of the Scientific Network on BSE-TSE 2019," 2019)(*Chronic Wasting Disease (CWD) | Prion Diseases | CDC*, n.d.).

CWD is a TSE that affects both confined and free-ranging Cervidae animals. CWD has been found in 14 US states, two Canadian provinces, and imported animals in South Korea since 1967. Surveillance for CWD, on the other hand, has been minimal across the world. Mule deer, white-tailed deer, black-tailed deer, Rocky Mountain elk, and Shira's moose are among the species impacted. Although intracerebral transmission of the scrapie agent has been demonstrated to cause CWD in elk, the cause of the illness remains unclear. It's possible that the afflicted populations have several PrP^{CWD} strains (Sigurdson & Miller, 2003). Horizontal transmission of CWD can be easily accomplished by contact with infected animals or environmental exposure, according to epidemiological and experimental findings (Sigurdson & Miller, 2003; Mathiason et al., 2009). Until recently, natural CWD transmission has not been observed in people who have been exposed to the afflicted region for an extended period of time and ingested venison, nor in domestic bovids such as sheep and cattle that share the environment with the infected cervids. Furthermore, when infected with the CWD agent, transgenic mice expressing human, ovine, or bovine PrP^{C} coding frames did not acquire the illness (Mathiason et al., 2009) .

Other cervids, such as red deer and reindeer/caribou, are vulnerable to CWD via intracerebral and oral routes, and may play a role in the continuing CWD pandemic in North America (Balachandran et al., 2010; Martin S et al., 2009). Cattle, sheep,

goats, ferrets, hamsters, bank voles, mink, raccoons, and squirrel monkeys are all intracerebrally transmissible to CWD (Sigurdson CJ, 2008).

In naturally and/or experimentally infected animals, PrP^{CWD} infectivity may be identified in the neurological system, lymphoreticular system, hematological system, skeletal and cardiac muscles, pancreas, fat, retina, and adrenal and salivary glands (Sigurdson & Miller, 2003; Sigurdson CJ, 2008) . There is virtually no tissue in affected cervids that is devoid of the CWD pathogen. Susceptible animals may acquire infectivity from their environments by eating grasses or drinking water contaminated with PrP^{CWD}, which affected cervids excrete, secrete, or deposit into the environment in the form of feces, urine, saliva, blood, placenta, and carcasses, even in the asymptomatic carrier state (Sigurdson & Miller, 2003; Sigurdson CJ, 2008).

The disease's prevalence in afflicted herds can range from 0.1 to 50 percent, and in extreme cases over 100 percent. CWD has been detected in the United States in areas far from the original endemic area, raising several questions, including whether infectivity was illegally transported to these areas in the form of tainted materials or infected animals, or in some other way, whether the scrapie agent has adapted to cause CWD through repeated natural passages into deer, and whether the PrP^{C} conversion in cervids is proficient. Given the uncertainty surrounding PrP^{CWD} transmission pathways, management options for disease eradication based on animal trade monitoring, quarantine, or enforced liquidation of afflicted herds or flocks may look less viable. As a result, therapeutic intervention aimed at (*CFSPH - The Center for Food Security and Public Health*, n.d.; *CFSPH - The Center for Food Security and Public Health*, n.d.) molecular pathways implicated in disease development may be a superior option for controlling CWD and scrapie outbreaks. (Imran & Mahmood, 2011).

The increased rate of CWD in free-ranging cervids, along with the poorer survival of sick animals, is considered to offer predators like mountain lions with an opportunity for selective predation. Local instabilities in ecological dynamics of food webs and nutrients recycling might result from such selective predation (Imran & Mahmood, 2011).

In affected cervids, chronic wasting of carcasses or weight loss, for which the condition is named "chronic wasting disease," is particularly frequent. CWD can also produce a rough, dry coat in these animals, as well as patchy retention of the winter

coat in the summer. Affected cervids, particularly elk, may exhibit additional mild signs such as lassitude, rapid mortality in deer following handling, a lowered head and drooping ears, and behavioral abnormalities such as fixated stare and loss of fear of people in preclinical or early clinical CWD. Tremors, teeth grinding, repetitive walking close to the enclosure's boundary, hyperexcitability with handling, excessive salivation due to difficulty swallowing, esophageal dilation or ruminal atony, regurgitation of ruminal fluid, polyuria, polydipsia. Before they die, many animals become very malnourished. CWD has incubation periods ranging from 16 months to 5 years, and both males and females are affected equally. After the start of clinical symptoms, death generally occurs within a year (*CFSPH - The Center for Food Security and Public Health*, n.d.).

The affected cervids' CNS displays intraneuronal vacuolation, degeneration and loss of neurons, widespread neuropil spongiosis, astrocytic hypertrophy and hyperplasia, and infrequent amyloid plaques on histological inspection. The thalamus, hypothalamus, midbrain, pons, medulla oblongata, olfactory tubercle, and cortex are the most common sites for sponge-like lesions. The dorsal motor nucleus of the vagus nerve, which is thought to be the earliest location of PrP^{CWD} accumulation, has the most consistent histological abnormalities and PrP^{CWD} immunohistochemistry staining. Importantly, severe injuries in the supra-optic and paraventricular nuclei, where anti-diuretic hormone is produced, may be responsible for clinical symptoms of polyuria and polydipsia, as well as low urine specific gravity in clinically dehydrated animals (*CFSPH - The Center for Food Security and Public Health*, n.d.).

Bovine spongiform encephalopathy

BSE (bovine spongiform encephalopathy) or the Mad Cow disease is a disease that affects cattle. It's one of the transmissible spongiform encephalopathies, a group of deadly neurodegenerative illnesses that afflict both people and animals (TSEs). They are caused by an aberrant form of prion protein, which is a type of cell protein (PrP). Only two instances(Mathiason, 2017;S. CJ, 2008)of BSE in animals other than cattle have been verified since the disease was first discovered in cattle: one goat in France and one goat in the United Kingdom (Dudas et al., 2021).

BSE was identified for the first time in 1986 the United Kingdom had an epidemic of classical BSE that was linked to the recycling of meat and bone meal used in feed supplements for cattle (Dudas et al., 2021). The European Commission

and EU Member States have established a comprehensive set of measures to mitigate the risk of BSE in the EU since 1989.

Tremors, gait irregularities, particularly in the hindlimb (ataxia), aggressive behavior, anxiety, and hyperreactivity to stimuli are all symptoms of BSE. In the brain, PrP^{Sc} build-up and spongiform vacuolation are common (Novakofski et al., 2005; Imran & Mahmood, 2011b). BSE prions can also be found in the spinal cord, retina, ileum, adrenal glands, tonsils, bone marrow, peripheral nerves, dorsal root ganglia, trigeminal ganglion, and thoracic ganglia at the end of the disease. Infectivity of BSE can be seen in brain tissues as early as two years after vaccination. There is no evidence of BSE prions in milk, sperm, or embryos, and there is little or no evidence of horizontal transmission, according to epidemiological and transmission research. Infected animals' progeny, on the other hand, have showed an elevated risk of disease development. The incubation period for BSE is 2 to 8 years, and the majority of BSE cases have been discovered in dairy cows that are 4 to 5 years old (Imran & Mahmood, 2011).

Classical BSE, H-type atypical BSE, and L-type atypical BSE are the three types of BSE. Classical BSE is the only kind that can be transferred to people via contaminated meat, resulting in variant Creutzfeldt-Jakob disease, which was first identified in 1996. The discovery of this oral transmission pathway sparked fears that people who ate BSE-infected meat may catch the disease as well. These were discovered in the United Kingdom, mostly in young individuals which were first identified in 1996 (Will et al., 1996; Dudas et al., 2021).

BSE surveillance testing is done with a variety of globally recognized test techniques. The protease-resistant characteristics of the BSE-associated prion protein are used in most of these approaches to distinguish and detect affected animals. A somewhat more sensitive approach for regular testing captures BSE-associated prion proteins using a PrP^{Sc} specific ligand. These techniques have been well verified for the many forms of BSE that have been identified, and they work well for monitoring in situations where samples are predominantly from higher-risk animals (Gray et al., 2011).

Feline and ungulate spongiform encephalopathy

FSE is a TSE that affects domestic cats and captive wild Felidae members. Since 1990, nearly 100 domestic cats, mostly from the United Kingdom, have been diagnosed with FSE, with one case each from Northern Ireland, Norway,

Liechtenstein, and Switzerland, as well as 29 captive wild cats, including 15 cheetahs, 4 lions, three each of ocelots, pumas, tigers, and an Asian golden cat. FSE instances have been reported in zoos in the United Kingdom, France, Australia, Ireland, and Germany. Except for a female cheetah and her cub born in France and another presumed to have acquired the infectivity in the Netherlands where she was born, all large zoo cats identified with FSE outside the UK originated from the UK. Because the majority of FSE cases coincided with the BSE outbreak, it was assumed that the disease was caused by cats eating PrPBSE-contaminated food. Mice injected with brain homogenates from FSE-positive cats and BSE-positive cattle developed a TSE with comparable neuropathological lesions profiles and incubation durations. In these mice, strain typing revealed a similarity between the FSE and BSE strains, confirming the theory that FSE is caused by BSE prions infection (Sigurdson & Miller, 2003,Eiden M et al., 2010, Bencsik A et al., 2009). However, in 1998, a domestic cat and his owner were found to be infected with a strain that was not related to PrPBSE. A phenotyp bifurcation was observed: the man had a phenotype similar to sCJD rather than vCJD, and the cat had a clinical phenotype separate from FSE. It's unclear if this happened by coincidence, if there was a horizontal transmission between the man and the cat, or if they both got the sickness from the same unidentified source . Another cat infected with the same strain and acquiring FSE from the environment has yet to be discovered. The FSE outbreak was quickly contained once the use of cow spleen and CNS tissue as pet food was prohibited. FSE has only been seen in cats older than two years (Sigurdson & Miller, 2003).

Human Prion Diseases

Prion diseases are transmissible protein misfolding disorders caused by the misfolding of a prion protein (PrP) encoded by the host. PrP is a protein of 253 amino acids (aa). Following transit to the endoplasmic reticulum, the first 22 N-terminal aa are removed from PrP, while the final 23 C-terminal aa are cleaved off after the insertion of the glycosylphosphatidylinositol (GPI) anchor, which aids the protein's attachment to the outer surface of cell membranes. PrP can be found in two different forms: a normal cellular prion protein called PrP^{C} and a pathogenic misfolded conformer called PrP^{Sc}. Both PrPC and PrPSc conformers are made up of base pairs 4,666,796-4,682,233 from the 16-kb single-copy *PRNP* gene on human chromosome 20 (20p13), which is located on the short (p) arm. The human *PRNP* has two exons, the second of which includes the whole open reading frame. In

secondary and tertiary structures, the aberrant PrP^{Sc} isoform varies from the normal PrP^{C} isoform but is not in the primary amino acid sequence. PrP^{C} is predominantly rich in alpha-helical contents, while PrP^{Sc} is predominantly rich in beta-sheet contents (Rafael L. et al., 2008, Imran & Mahmood, 2011a, Z. R et al., 2000). The PrP^{Sc} isoform is particularly resistant to proteolysis and destruction by traditional chemical and physical cleaning or disinfection methods due to this structural difference. PrP^{C} is soluble in non-denaturing detergents and is fully destroyed by proteases, unlike PrP^{Sc} (Rafael L. et al., 2008; Imran & Mahmood, 2011; *Chronic Wasting Disease (CWD) | Prion Diseases | CDC*, n.d.).

Sporadic Cruetzfeldt- Jacob disease (sCJD)

Sporadic Cruetzfeldt-Jacob disease caused for 85% of all CJD cases, with an annual global incidence of 1-2 cases per million people (Mead S et al., 2003). It affects in both genders with get peak age of onset between 55 and 75 years. Some oldest (above 90 years) and younger (below 20 years) cases have also been reported. Rapidly progressing dementia, cerebellar dysfunction, including muscular incoordination, visual, speech, and gait impairments are among the clinical signs. Dementia is a primary symptom followed by induced myoclonia or spontaneous. During the disease course, symptoms of extrapyramidal dysfunction with reflexes and pyramidal, spasticity, tremors and rigidity, and behavioral changes with agitation, depression and confusion may also be observed. Finish of the disease course, mostly the patients go in a state of akinetic mutism (they become unresponsive to exterior stimuli) (Vitale et al., 2016).

The presence of sparse PrP^{Sc} distribution and spongiform changes in CNS of sCJD patients are the distinguishing of the neuropathology of the disease. In 5-10% of instances, amyloid plaque deposits might be seen. Different sCJD variants have been identified based on clinicopathological characteristics. The amaurotic or Heindenhain variant is characterized by rapid progression of myoclonus, dementia, and visual disturbances such as hallucinations, cortical blindness, visual agnosia, and as well as a short disease duration; the Brownell and Oppenheimer variant is characterized by early and predominant cerebellar ataxia and relatively late dementia; the thalamic form is characterized by dementia and movement disorders (Cornelius JR et al., 2009; Parchi P et al., 2011). Cases with sCJD can be categorized as potential, likely, or definite based on clinical, biological, electrophysiological, and neuropathological data. Characterized by a large number of cells in the CSF,

extensive white matter destruction, and a long illness course. Rapidly progressing dementia with at least two of the following symptoms: myoclonus, cerebellar or visual symptoms, pyramidal or extrapyramidal indications, akinetic mutism, and a disease course of less than two years are all possible instances. When a characteristic periodic EEG or increased levels of 14-3-3 protein in CSF are found in possible cases, they are considered probable cases (Imran & Mahmood, 2011a). The presence of spongiform alterations or PrP^{Sc} reactivity in the brain defines definitive instances. Symptoms of sCJD can also be found in illnesses such as Alzheimer's disease, diffuse Lewy body disease, or frontal dementia, paraneoplastic syndrome, tumor, or stroke, as well as problems caused by toxins, nutrition, metabolism, or infection. As a result, differential diagnosis for potential and likely cases of sCJD is required. In certain situations, a computed tomography (CT) scan may reveal signs of moderate brain atrophy. In more than half of cases, cranial magnetic resonance imaging (MRI) can identify brain atrophy by revealing strong signals on T2, flair, or diffusion-weighted sequences in the basal ganglia or cerebral or cerebellar cortex (Schmitz et al., 2017). Electroencephalographic (EEG) signals are changed in almost all instances of sCJD. Slow wave activity remains consistent over time and deteriorates as the illness progresses. In instances of sCJD, EEG shows pseudo-periodic sharp wave complexes of 1 Hertz. Repeated EEG recordings are necessary to overcome the transitory nature of periodic sharp wave complexes.

Variations in *PRNP* get a major effect on the clinicopathological and molecular manifestations of sCJD. *PRNP* polymorphisms in both regulatory and coding regions have been linked to a higher risk of illness development. 2007; Palmer MS et al., 1991). Homozygozity for the M129V polymorphism is a significant risk factor for sCJD development (Palmer MS et al., 1991, Windl O et al., 1996). In Japanese, homozygosity at another *PRNP* polymorphism E219K is also a risk factor for the development of sCJD (Shibuya S et al., 1998a, 1998b). Only Asian and Pacific groups have E219K, which are more homozygous for methionine at codon 129 than Western populations (Soldecila M et al., 2003). Ineffective interaction between heterologous PrP^{C} molecules generated by different *PRNP* alleles (129 M and 129 V) could limit PrP^{C} to PrP^{Sc} conversion. Any major hurdle in the prion proliferation process is thought to inhibit the disease's development (Palmer MS et al., 1991). Different prion strains are responsible for the clinical phenotypic variability found in sCJD patients. The pattern and ratios of PrP^{Sc} fragments on a Western blot

characterize a strain biochemically. Three M129V genotypes (129 MM, 129 MV, and 129 VV) and two strain types (type 1 and type 2) may be combined into six distinct combinations (MM1, MV1, VV1, MM2, MV2, and VV2) that closely match different sCJD clinical presentations. Three alternative strain categorization methods have been developed based on these six combinations (Parchi P et al., 2011, Brandner. S, 2011,Wadsworth JD & Collinge J, 2011).

Inherited prion disease

Inherited prion diseases are caused by mutations in the *PRNP* gene, which codes for the prion protein. More than thirty variants have been identified, resulting in a wide spectrum of clinical symptoms between and within families (Collinge J, 2001). The importance of variables other than the prion protein sequence in prion disease pathogenesis, including as environmental and epigenetic factors, is highlighted by the fact that a single *PRNP* mutation may cause many disease manifestations. DNA sequencing of *PRNP* from blood samples can be used to provide a pre-symptomatic genetic diagnosis of hereditary prion disease. Fatal familial insomnia (FFI), a rare condition seen in numerous Italian families, is an example of inherited prion disease.

Fatal familial insomnia (FFI)

Known as thalamic dementia, fatal familial insomnia (FFI) is an autosomal dominantly inherited human prion disease that was renamed in 1986. FFI is caused by the *PRNP* mutation D178N, which is related to the *PRNP* polymorphism M129V's methionine. When coupled to valine at codon 129, the identical mutation causes a variety of f/gCJD phenotypes (Capellari S et al., 2011). FFI has been recorded 100 cases in almost 40 families in Italy, Germany, Austria, Spain, the United Kingdom, France, Finland, the United States, Australia, Japan, China, and Morocco (Baldin E et al., 2009). FFI affects both men and women equally, with no substantial differences between the 129 MM and 129 MV genotypes. In 129 MM individuals, however, the illness duration may be substantially shorter. FFI patients range in age from 20 to 72 years old, with an average of 49 years, and can survive for 8 to 72 months following the beginning of the disease, with an average of 18.4 months. Insomnia or disrupted sleep are common clinical complaints. There may also be myoclonus, ataxia, dysarthria, dysphagia, and pyramidal symptoms, as well as autonomic hyperactivation. Clinical symptoms severity and sequence may differ

between the 129 MM and 129 MV genotypes. In 129 MM patients, insomnia, myoclonus, and autonomic dysfunction are more common, whereas ataxia, dysarthria, and seizures are more common in 129 MV subjects (Belay ED, 1999, Capellari S et al., 2011, Montagna P et al., 2003, Gambetti P et al., 2003).

Polysomnography is extremely useful in determining the cause of the illness. It demonstrates insomnia by demonstrating a significant reduction in sleep duration and a disordered transition between sleep phases. The 129 MV genotype has been shown to lessen the severity of these atypical sleep symptoms. In contrast, positron emission tomography indicated that the 129 MV genotype is linked to more extensive hypometabolism in the thalamus and cingulate cortex (PET). As a result, PET might be another helpful technique in the diagnosis of FFI. Neuronal loss and astrogliosis occur primarily in the anterior and dorsomedian thalamic nuclei in all instances and in the inferior olives in most cases with little PrP deposition with subsequent cerebral cortical and cerebellar involvement. PrP deposits, on the other hand, may be more visible in the cerebellum's molecular layer, which has a stripe-like pattern, and in the subiculum entorhinal area (Belay ED, 1999; Capellari S et al., 2011, Montagna P et al., 2003; Gambetti P et al., 2003).

Kuru

Kuru has been the first human prion illness to be proved to be transmissible to chimps by intracerebral injection of kuru patients' brain homogenates, also known as prions (the infectious protein particles) . Kuru has only been found among the Fore linguistic group in the Eastern Highlands of Papua New Guinea, as well as the adjacent peoples with whom they intermarried. As a gesture of respect and grief, these tribes would devour the bodies of their kin (ritualistic cannibalism). Women and young children of both sexes were more exposed to danger materials such as brain and viscera than adult men, who tended to eat muscles first. At its peak, the kuru pandemic killed 1-2 percent of the population. Adult women were especially scarce in some communities. The prevalence of the disease began to drop gradually when Australian authorities put a prohibition on ceremonial cannibalism in the mid-1950s. Kuru was initially noticed about 1920 and was first brought to Western medicine in the late 1950s. Western experts were quickly able to establish ritualistic cannibalism as the disease's cause. Older kuru patients who were exposed to the virus prior to the prohibition are still visible. In such patients, the incubation time for kuru

would be more than 50 years (Liberski PP & Brown P, 2009; Mead S et al., 2003, Brandner S et al., 2008, Mead S et al., 2009). Kuru has put a lot of pressure on *PRNP*, particularly at codons 127 and 129, for selections repression in the susceptible Fore groups. Heterozygosity at these *PRNP* codons is a kuru resistance factor, and it is seen in high numbers among kuru epidemic survivors (Liberski PP & Brown P, 2009, Mead S et al., 2009).

Kuru has three clinical stages: ambulant (still able to walk), sedentary (only able to sit up), and terminal (no longer able to move) (unable to sit up independently). These stages may be preceded by an ill-defined prodromal period marked by headache and discomfort, mainly in the joints of the legs. Cerebellar ataxia, tremors, and choreiform and athetoid motions are all obvious clinical symptoms. The illness was termed "kuru" because of the sign of shivering that was exacerbated by cold. Dementia sickness, the most conspicuous clinical characteristic of sCJD, can also occur in certain instances, but only in the late stages of the disease. Spongiosis, neuronal loss, and astrocytic microgliosis are all neuropathological characteristics that can be seen in the CNS, mainly in the grey matter. PrP^{Sc} deposition is exclusively seen in the CNS (Liberski PP & Brown P, 2009; Brandner. S et al., 2008).

Iatrogenic CJD (iCJD)

The first case of iatrogenic CJD (iCJD) was reported in 1974 in a person who had a cadaveric corneal transplant from a CJD patient (Duffy P et al., 1974). Since then, the use of stereotactic intracerebral EEG needles or neurosurgical tools, cadaveric dura mater grafts, and intramuscular injections of contaminated cadaveric pituitary-derived human growth hormone (hGH) and gonadotrophin hormone have been linked to iatrogenic transmission of CJD. Treatment with hGH and Lyodura grafts made by B. Braun Melsungen AG of Germany and processed before May 1987 is responsible for the majority of iCJD cases. The majority of CJD cases connected to hGH therapy occurred in France, while those linked to dura mater transplants occurred mostly in Japan (Belay ED, 1999, Nozaki I et al., 2010 ,Will RG, 2003). About 30,000 children have been treated with hGH globally between the late 1950s and 1985, with the total proportion of CJD cases from the treated population estimated to be around 1/100. Batch processing is thought to be to blame for the potential PrP^{Sc} contamination of pituitary-derived hGH and dura mater transplants. Prior to the development of recombinant hGH, it was common procedure

in the pharmaceutical industry to extract the hormone from 5,000 to 20,000 cadaveric pituitary glands in a single batch. Contamination may have occurred due to the inclusion of a few pituitary glands from unknown CJD victims in the batch. Since 1987, when recombinant hGH became available and independent processing of individual dura mater grafts was introduced, the likelihood of future iCJD cases appears to be quite low. However, with lengthy incubation periods, a surge in the frequency of iCJD patients may develop (Belay ED, 1999, Will RG, 2003). The clinicopathological characteristics of CJD associated with hGH therapy are similar to those of kuru. The 129 MM homozygozygosity is a risk factor, and incubation durations can range from 4.5 to more than 25 years, with an average of 12 years (Belay ED, 1999; Will RG, 2003; Collinge J et al., 1991). The clinicopathological characteristics of CJD associated to dura mater neurografting or the use of neurosurgical tools, on the other hand, are similar to those of sCJD. The average length of sickness is 18 months, while incubation durations range from 1.5 to 18 years, with an average of around 6 years. In Japan, the chance of contracting iCJD is believed to be around 1/3000 Lyodura graft patients (Will RG, 2003).

Role of the PRNP Gene for Animal

Ovis aries (Sheep)

Molecular techniques based on polymerase chain reaction (PCR) and DNA sequencing technologies have been widely using to detect the *PrP* gene variations. In sheeps, various polymorphisms within the *PrP* gene are associated with different phenotypic expression of prion diseases such as incubation period, pathology and clinical manifestations. Although more than 30 polymorphisms have been identified, only a few of them are closely related to show resistance susceptibility to classical Scrapie. Three *PrP* gene variations have a particular strong relation with both natural and experimental formation. These substitutions are valine (V) to alanine (A) on the codon 136, histidine (H) to arginine (R) on the codon 154 and arginine (R) to glutamine (Q) on codon 171. Only five of 12 haplotype forms are common. These are 136-, 154-, 171-, ARR, ARQ, VRQ, AHQ and ARH haplotypes respectively. ARR haplotype has been asscoaited with resistance, controversially, VRQ haplotype has been associated with susceptibility to scrapie. Breeds like Cheviot and Suffolk are more susceptible to scrapie than other breeds (Straiton E., 2001).

Capra aegagrus hircus (goat)

At least 47 amino acid substitutions and 19 silent variations have been determined in the goat PRNP genein several countries (Ricci et al., 2017). On the other hand, serine at codon 146 is present at a frequency of 16.5% in Capra aegagrus circus (Damascus goat) of Cyprus but absent in Derivata di Siria, another Damascus related goat breed in Sicily, that showed conversely a 15% frequency of the resistant variant p. (Gln222Lys) ("Scientific and technical assistance on the provisional results of the study on genetic resistance to Classical scrapie in goats in Cyprus," 2012) . The PRNP gene p.(Asn146Ser) substitution have been determined as an important genetic factor that shows resistance to scrapie. (Vitale et al., 2019). Recently, the effect of these variables on prion infectivity and disease outcomes has been confirmed by experimental infections (Papasavva-Stylianou et al., 2017).

The goats, PrP amino acid polymorphisms at codons 21 (V → A), 23 (L → P), 49 (G → S), 142 (I → M), 143 (H → R), 154 (R → H), 168 (P → Q), 220 (Q → H), 240 (S → P) and "silent" mutations at codons 42 (A →G), 107 (G→ A), 138 (C → T) and 207 (G → A) have been described (Papasavva-Stylianou et al., 2007a). Genotypes with susceptibility and resistance in goats are shown in Table 3.

Riccie et al., (2017) have also identified curicial polymorphisms which they have predisposition to resistance for scrapie. These alleles are: S127, M142, R143, D145, D146, S146, H154, Q211 and K222 goats. (Ricci et al., 2017). K222, D146 and S146

K222 genotypes are shown in Table 4 by European goat species.

Table 3.

Genotypes with susceptibility and resistance in goats

Position	Genotype	Susceptibility	Resistance
142	MM		++
	IM		+
	II	+	
146	SS		+++
	NS		++
	NN	+++	
	ND		
	DD		++
	DS		+++

154	HH		++
	RH		+
	RR	+	
211	QQ		++
	RQ		+
	RR	+	
222	KK		++++
	KQ		+
	QQ	+	
240	PP		+++
	PS		+
	SS	+	

(Wilfred Goldmann et al., 2011; “Scientific and Technical Assistance on the Provisional Results of the Study on Genetic Resistance to Classical Scrapie in Goats in Cyprus,” 2012; Castañeda-Bustos et al., 2014; Goldmann et al., 2016a; Georgiadou et al., 2017; W. Migliore et al., 2020).

Table 4.

S146/D146 and K222 geneotypes have been described in the literature, and their frequencies have been published in the EU.

Country	Breed	Frequency
146S or 146D		
UK	Boer	24.5-35.5
Netherlands	Boer	31
	Nubian	7.1
Greece	Local/crossbred	3
South Cyprus	Dmascus and related breeds	16.5
222K		
UK	Toggenburg	1.9
Netherlands	Saanen	1.9
	Toggenburg	29.5
Greece	Local/crossbred	5.6
France	Saanen	4.9
Spain	Saanen	1.2
	Local breed	6.4
	Alpine	0-0.03
Italy (North)	Camosciata	2.4
	Saanen	3
	Roccaverano	4.3
	Valdostana	1.3
Italy (South)	Garganica	17.2
	Jonica	7.3
	Southern crossbred	22.5

	Girgentana	18.7
	Rossa Mediterranea	12.7
	Argentata dell'Etna	16.3
	Aspromontana	10.3
	Cilentana	18.2

EU Regulations about PRNP genetic testing

EU legal background: TSE legislation was enacted in the EU as a result of the discovery of BSE in cattle in the UK in the late 1980s. The first TSE-related legislation was passed in 1989, and it prohibited the export of some live cattle from the United Kingdom to the other member states. Other measures were gradually implemented in the EU, particularly in relation to cattle BSE, to prevent and limit the disease's spread to ruminants (e.g. feed bans, measures applied to suspected and confirmed TSE cases, trade restrictions) and humans (e.g. removal of specified risk material (SRM) from animals slaughtered for human consumption) ("Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats," 2014) .

Scrapie surveillance systems in sheep and goats have evolved throughout time. Initially, only scrapie-like symptoms were tested, but in 2002, sample-based surveys (active surveillance) were implemented in two additional categories of small ruminants (Regulation (EC) No 1248/2001): healthy animals slaughtered for human consumption (SHC) and animals not slaughtered for human consumption (fallen dead on farm) (NSHC) over 18 months of age, to be tested using a rapid screening test and a confirmatory test ("Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats," 2014a; "The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021) .

Information to be reported in the European Union TSE Database: In the context of the monitoring program, MSs report the number of ovine and caprine animals tested within each subpopulation, as well as the sample selection procedure and the results of the fast and confirmatory tests. MSs report the number of suspected TSE cases that have been placed under official movement restrictions, the number of suspected TSE cases that have been subjected to laboratory examination per animal species, including the results of rapid and confirmatory tests, and the number of flocks where suspected TSE cases in ovine and caprine animals have been reported and investigated. Finally, MSs describe the geographical distribution of positive CS, AS, and BSE cases, including the country of origin, the animal's year and, if feasible,

the month of birth, the findings of primary molecular testing for scrapie and BSE cases, and, for sheep, the genotype and, if possible, the breed.

The European Commission collected this information from MSs in various methods over the years until a standardised database and submission mechanism was developed in 2006, allowing for the acquisition of more data. MSs transmit national data to the EU TSE database directly through two types of reports: 'monthly reports' and 'case reports,' for both sheep and goats (“Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats,” 2014a) .

MSs submit monthly reports that contain aggregated data on all TSE tests performed in sheep and goats, including data on the animals tested (age, target group), the flock (whether the flock is under official control due to TSE cases or not, and geographical location), the first test (which rapid test was used as the initial screening test), and the total number of animals tested, including positive, negative, and asymptomatic animals.

Regulation (EC) 894/2017 revised the TSE Regulation in 2017 with reference to representative genotyping efforts in ovine populations, as stated in the 2018 EUSR on TSE (EFSA, 2019). For MS with an adult sheep population of more than 750,000 animals, the TSE regulation no longer requires genotyping a minimum sample of 600 animals, and for other MS, a minimum sample of 100 animals. The new rules mandate genotyping every three years with a minimum sample size of 1,560 ovine animals or at a frequency and sample size chosen by the MS based on a set of criteria (“The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020,” 2021).

MSs send yearly 'case reports,' which contain specific individual information about positive TSE cases detected in sheep and goats. Scrapie type, unique national case number, month and year of birth, country of origin, breed and genotype), flock of origin of the animal (national flock identification number, geographical location), and tests performed (i.e. type and result of rapid test(s) and confirmation method(s) used to identify the case, discriminatory method(s) used to differentiate CS from BSE) should all be submitted (“Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats,” 2014a) . Folk numbers reported by country are shown in Table 5.

Table 5.
The number of flocks for whom suspected cases of ovine and caprine animal disease have been reported and investigated in accordance with the law ("The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021).

Country	Sheep	Goat
Austria	0	0
Belgium	0	0
Bulgaria	6	4
Croatia	7	31
Cyprus	0	0
Czechia	12	8
Denmark	0	1
Estonia	0	0
Finland	3	0
France	0	1
Germany	0	0
Greece	0	0
Hungary	0	0
Ireland	0	0
Italy	2	0
Latvia	1	0
Lithuania	0	0
Luxembourg	0	0
Malta	0	1
Poland	0	0
Portugal	0	0
Romania	2	0
Slovakia	0	0
Slovenia	14	5
Spain	1	1
Sweden	0	0
The Netherlands	0	0
Total EU27	**48**	**53**
The United Kingdom	0	1
Total EU27 + UK	**48**	**54**
Bosnia and Herzegovina	0	0
Switzerland	0	0
Iceland	6	1
Montenegro	0	0
North Macedonia	0	0
Norway	2	0
Serbia	0	0
Total other non-EU	**8**	**1**
Total	**56**	**55**

Cyrus Regulations about PRNP genetic testing

Since 1985, 1019 Scrapie epidemics have been identified in Southern Cyprus. It has been noted that the majority of outbreaks involve mixed flocks of sheep and goats. In 2002, 2003, 2004, and 2005, there was a rapid increase in the frequency of outbreaks, and the breeding program began in 2004. As a result of the breeding program implemented to all sheep-rearing herds, resistance has been observed to grow in sheep. In 2009, a program to develop goats resistant to Scrapie was launched in Cyprus (EU report, 2014). Between 2009 and 2014, 722 Scrapie-infected flocks were in operation. These surveys account for 22.6% of all operational sheep and goat farms. This is due to the fact that past outbreaks have subsided and new outbreaks have increased the number of known infected herds (EU report, 2014). Utilizing genetic breeding techniques in Chios sheep, the Agricultural Research Institute (ARI) has successfully launched a project to combat scrapie on the island of Cyprus. In Athalassa, where a natural park is located in Nicosia, South Cyprus, there was a Chios sheep unit. This herd has been converted into a nucleus herd comprised of Scrapie-resistant genotypes. All of the sheep in the herds have the ARR/ARR genotype, which confers complete disease resistance. This sheep has been bred and supplied to farmers in Athalassa Park. At the end of each lambing season, farmers are given a large number of young rams and females to boost the genetic density and productivity of their herds, as well as the frequency of resistant genotypes in the Cypriot sheep population. More than 4,700 Scrapie-resistant sheep have been distributed to farmers for growth and milk production during the past 15 years. Midway through 2008, a research program to turn the Cyprus Damascus goat breed into 300 adapt-universal-resistant genotyping nuclei by means of targeted mapping was initiated. This program enables ARI to increase the quantity of animals distributed to farmers. These animals have superior genetic stigma and are also regarded as disease-resistant. In this context, ARI has made a significant contribution to the fight against scrapie in the Cypriot population (Republic Of Cyprus Agricultural Research Institute, 2018).

Evidence from investigations of Scrapie control in goats in Cyprus suggests that the S146 and D146 alleles provide resistance to Scrapie (Papasavva-Stylianou et al, 2007). Animals confiscated since 2009; animals with dubious clinical signs include those that were not slaughtered for human food due to their PrP genotype or age. The number of animals has been continuously declining as a result of the adoption of the

breeding program for durability in sheep. In contrast, the number of confiscated goats has been rising. Under the sheep breeding program, rams without an ARR / ARR alleline are not utilized as studs. Ram lacking ARR alleles are either culled or dealt with (FAO, 2013). Cyprus's breeding program for its eradication program is unique among EU member states.

In spite of the lack of an official eradication effort in Northern Cyprus, an eradication program was implemented in 2003, killing roughly 3000 sheep and compensating the farmers. Due to economic challenges, this situation could no longer be maintained. Unofficially, farmers in Northern Cyprus have purchased rams with the ARR/ARR genotype from farms in Southern Cyprus. Depending on this factor, the incidence of Scrapie illness in Northern Cyprus is decreasing.
Genetic identification of TRNC sheep breeds for resistance to Scrapie disease was conducted in sheep and goats in Northern Cyprus in collaboration with the Food and Agriculture Ministry of Livestock of Turkey and the Food and Agriculture Ministry of TRNC. 16 percent extremely resistant ARR / ARR, 21 percent resistant single allele ARR, and 62 percent partially resistant genotypes were detected in the risk category, per project data (TAGEM, 2012).

The work in this thesis

Blood will be taken from Alzheimer's Disease patients and individuals which do not show any symptoms of AD considering as health group. Sheep and goat samples were identified by applying morphometric measurement, and blood samples were taken after they were defined. This thesis is approved by the Near East University Animal Ethics Committee (No: 2019 / 05-75). The thesis budget is granted by NEU, however money transferring process is still pending. By then sample collection and DNA isolation analysis by conducting in the DESAM Institute Molecular Medicine Laboratory.

CHAPTER II

Materials And Methods

Materials

Suppliers

Thermo Scientific (Pittsburg, USA), Sigma-Aldrich (Poole, UK), Bio-Rad (California, USA), Qiagen (Hilden, Germany), Intron (Seoul, South Korea), Illumina (San Diego, USA), Leica (Wetzlar, Germany), Macrogen (Seoul, South Korea), Invitrogen (California, USA).

Isolation Kits

Genomic DNA isolation from the whole sheep and goat animal blood was done with PureLink™ Genomic DNA Mini Kit with ThermoFisher Scientific.

Animal subjects

100 farm animal subjects from seven herds participated in this study. Ovis aries aries (sheep) and Capra aegagrus hircus (goat) different species used.

Ovis aries aries (sheep) samples

A total 50 sheep blood samples were collected from six different farm locations (Dikmen, Boğaz, Düzova (two different farm), Vadili and Büyük Kaymaklı (Ercan)). Sheep samples are Awassi (n=15), Sakız (n=12) and hybrids (n=23).

Figure 1

Blood sampling from sheep

The animal has been prepared for shearing. A patch approximately 4 inches broad and 8 inches long is shaved using electric shears. The animal is held by its lower jaw and its head is tilted at a 30-degree angle to the side so that the vein can be easily accessed. The vein is located by applying pressure with the thumb or fingers beneath the shaved area's midpoint. Pressure will force the vein to protrude and become visible. The collecting site is cleansed with three scrubbing of 70 percent alcohol and betadine that are alternated. Before placing the needle into the vein, the plunger is retracted to break the seal on a new syringe, and then the plunger is fully depressed to expel any air drawn into the syringe. The needle is placed into the vein using a 6 cc syringe, a 20-gauge, one-inch or shorter needle, and a vacutainer. The syringe is slowly retracted to determine whether the needle is in the vein. It may be necessary to gently move the needle until blood can be easily drawn back into the syringe. Once the sample has been acquired, the vein is decompressed and the needle is removed. Hemostasis is achieved by exerting hard pressure to the site of blood collection with sterile gauze.

Capra aegagrus hircus (goat) samples

A sample of whole blood with EDTA was collected from V. jugularis for genetic analysis. A total of 100 small ruminant (50 sheep and 50 goat) blood samples were collected from seven different farm locations **(**Dikmen, Boğaz, Düzova (two different farms), Vadili, Akdeniz and Büyük Kaymaklı (Ercan)). Sampling was carried on clinically healthy goats belonging to Native Cyprus Goat (n=20), Damascus (n=16) and hybrids (n=14). The same procedure for bloodletting in sheep was applied.

Figure 2

Some phenotypic profiles of Cypris native hair goat (CNHG)

Data Collection and Study Design

While choosing the examples; We made sure that the farms did not come into contact with any farm from Southern Cyprus**. Samples were taken from farms with specified breeds. We chose mixed farms, excluding native goats.**

A total of 50 different sheep blood samples were taken in Northern Cyprus from five different places, including Dikmen (n=6), Sutluce (n=8), Vadili (n=10), Duzova (n=11)and Buyuk Kaymakli (Ercan) (n=11). Clinically healthy sheeps from Chios (n=10), Awasi (n=15), and hybrids (n=25) were used for sampling. The regions where the samples were taken, their ages and races are shown Table 8.

Table 8.

Blood sampling farms, age, Ovis aries aries race and gender

Region 1 : Dikmen		**Date: 11.06.2019**			
No	**Ear no**	**Age**	**Race**		**Gender**
S.1.1	NC040437141	1	SAKIZ-hybrid	Awassi	Ram
S.1.2	NC040437151	1	SAKIZ-hybrid	Awassi	Ram
S.1.3	NC040437145	2	SAKIZ-hybrid	Awassi	Ram
S.1.4	NC 032372561	2	SAKIZ-hybrid	Awassi	Ram
S.1.5	NC 034810840	3	SAKIZ-hybrid	Awassi	Ram
Region 2: Düzova		**Date: 17.07.2019**			
No	**Ear no**	**Age**	**Race**		**Gender**
S.2.1	RAM 1	11 Month	SAKIZ-hybrid	Awassi	Ram
S.2.2	RAM 2	11 Month	SAKIZ-hybrid	Awassi	Ram
S.2.3	RAM 3	11 Month	SAKIZ-hybrid	Awassi	Ram
S.2.4	861897	3 Age	SAKIZ-hybrid	Awassi	Sheep
S.2.5	861297	4 Age	SAKIZ-hybrid	Awassi	Sheep

S.2.6	902467	3 Age	SAKIZ-hybrid	Awassi	Sheep
S.2.7	902686	3 Age	SAKIZ-hybrid	Awassi	Sheep
S.2.8	902521	2 Age	SAKIZ-hybrid	Awassi	Sheep
S.2.9	861936	4 Age	SAKIZ-hybrid	Awassi	Sheep

Region 4: Büyük Kaymaklı (Ercan)		**Date: 26.09.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
S.4.5	NC036903256	-	Awassi	Ram
S.4.6	531026	-	Awassi	Sheep
S.4.7	NUMARASIZ	-	Awassi	Sheep
S.4.8	268131	-	Awassi	Sheep

Region 5: Boğazköy		**Date: 20.08.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
S.5.1	383816	2	Awassi	Ram
S.5.2	701056	2	Awassi	Sheep
S.5.3	598529	1,5	Awassi	Ram
S.5.4	90865092	3	Awassi	Sheep
S.5.5	328271	1	Awassi	Ram
S.5.6	O86986	2	Awassi	Sheep
S.5.7	679718	2	Awassi	Sheep
S.5.8	701206	3	Awassi	Sheep
S.5.9	NC036701034	1,5	Awassi	Sheep
S.5.10	701090	2	Awassi	Sheep
S.5.11	KOÇ NUMARASIZ	4	Awassi	Ram

Region 6: Vadili		**Date: 27.09.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
S.6.1	NC038943165	1	SAKIZ	Ram
S.6.2	943171	2	SAKIZ	Sheep
S.6.3	396931	2	SAKIZ	Sheep

S.6.4	O35747	2	SAKIZ	Sheep
S.6.5	794741	1,5	SAKIZ	Sheep
S.6.6	794785	2	SAKIZ	Sheep
S.6.7	O35871	4	SAKIZ	Sheep
S.6.8	943232	1,5	SAKIZ	Sheep
S.6.9	NC036035732	4	SAKIZ	Ram
S.6.10	O35493	3	SAKIZ	Sheep

Region 7: Düzova		**Date: 12.10.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
S.7.1	NC038292498	2	SAKIZ-hybrid Awassi	Sheep
S.7.2	NC032303046	3	SAKIZ-hybrid Awassi	Sheep
S.7.3	NC028567520	4	SAKIZ-hybrid Awassi	Sheep

Region 8: Sütlüce		**Date: 10.11.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
S.8.1	-	1	SAKIZ-hybrid Awassi	Sheep
S.8.2	-	3	SAKIZ-hybrid Awassi	Sheep
S.8.4	-	5	SAKIZ-hybrid Awassi	Sheep
S.8.5	-	4	SAKIZ-hybrid Awassi	Sheep
S.8.6	-	2	SAKIZ-hybrid Awassi	Sheep
S.8.7	-	2	SAKIZ-hybrid Awassi	Sheep
S.8.8	-	3	SAKIZ-hybrid Awassi	Sheep

Methods

Animal Genomic DNA Extraction

Using an Invitrogen DNA isolation kit (Thermo Scientific, UK), DNA was extracted from whole blood and EDTA-treated tubes from every goat tested.

Dna measured

Nanodrop (NanoDropTM 2000/2000c, Thermo, UK) was used to quantify DNA concentrations.

Primer design

The coding region of the PrP gene (GenBank accession number M31313, located between nucleotides 24 and 912) was amplified with a specifically designed primer pair with the help of the Primer Blast Tool from NCBI (National Center of Biotechnology Information).

Table 6.

Information on designed premers:

Gene	**Primer**	**PCR**	**Sequence**
PRNP For sheep	F	**5'-TCTTACGTGGGCATTTGATG-3'**	**5'-CAACCGCTATCCACCTCAG-3**
	R	**3'AACAGGAAGGTTGCCCCTAT-5',**	**3'-AGCCTGGGATTCTCTCTGGT –5'**
PRNP For goat	F	**5' -ATGGTGAAAAGCCACATAGG-3'**	**5'-AACCAACATGAAGCATGTGG-3'**
	R	**-3'-TATCCTACTATGAGAAAAATGAGG-5'**	**3'-GAT-AGTAACGGTCCTCATAG-5'**

Sequencing

Sequencing was performed using Macrogen. For each sample, the requireed amount of DNA was as 20- 50 ng/ul and the sample amount prepared 20 µl.

CHAPTER III
Results of Sheep PRNP Gene Sequencing

Introduction

The BSE pandemic in the United Kingdom in the 1990s piqued interest, as did evidence BSE-contaminated meat causes variant CJD in humans (Greenlee, 2019). The general mechanism of protein misfolding diseases is that misfolded proteins stimulate the refolding of normal cellular protein, which accumulates and causes brain degeneration (Spiropoulos et al., 2011). It is the host-encoded cellular prion protein (PrP^{C}) that is misfolded into a pathogenic form in prion disorders (PrP^{Sc}). There is no new PrP^{Sc} creation in the absence of PrP^{C}, such as in PRNP knockout or PrP^{Sc} depletion. Prion illnesses differ from other protein misfolding disorders in that they might potentially spread to vulnerable persons upon natural exposure (Greenlee, 2019). Scrapie, also known as transmissible spongiform encephalopathy (TSE), is a deadly neurological illness that affects sheep and goats (Zeineldin et al., 2021). Scrapie was first discovered in sheep and goats in the United States in 1947 and 1969, respectively, and has since become a major health concern in both small ruminants. Scrapie's presence in the World costs around $20 million per year due to major production losses, export losses of sheep and goat breeds, including genetic materials, and increased carcass disposal costs (Greenlee, 2019; Zeineldin et al., 2021).

In the report published every year by the European Union, genotypes were classified according to their resistans or susceptible properties. Table . genotypes are shown according to their resistans or susceptible properties.

Table 8.

The National Scrapie Plan (NSP) and the three tiers of report groups were used to classify the genotypes of the sheep prion protein PRNP gene ("The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021).

NSP group	Genotype	Comment	Report group
NSP1	ARR/ARR	Genetically most resistant	Resistant

NSP2	ARR/ARQ; ARR/ARH; ARR/AHQ	Genetically resistant	Semi-resistant
NSP3	ARQ/ARQ	Genetically little resistant (ARQ/ARQmay be scientifically reviewed)	Susceptible
NSP3 Other (NSP3O)	AHQ/AHQ; ARH/ARH; ARH/ARQ; AHQ/ARH; AHQ/ARQ		Susceptible
NSP4	ARR/VRQ	Genetically susceptible	Susceptible
NSP5	ARQ/VRQ; ARH/VRQ; AHQ/VRQ; VRQ/VRQ	Genetically highly susceptible	Susceptible

The susceptible genotype groups accounted for 541 (97%) of the 558 cases of CS in sheep with NSP genotype reported in the EU27 and the UK in 2020 (91.9 percent of the total CS caseload) (NSP3, NSP3O NSP4 and NSP5). This is comparable to prior years, when the vulnerable groups accounted for nearly 98.7% of all CS cases with known genotypes. All CScases reported by Iceland in the other non-EU reporting nations were from susceptible genotype groups or other non-NSP genotypes. Romania has reported one instance of CS in a sheep with the ARR/ARR genotype (NSP1) in 2020, a very unusual event that Spain recorded in 2019. The same genotype groups (NSP3, NSP3O, NSP4, and NSP5) accounted for 49.5 percent (46/93) of all cases with known genotype (46.9% of the total AS caseload) in ovine AS patients, which is quite comparable to 2019. Following changes in legislation that took effect in 2018, nine MS in the EU27 and the UK genotyped a random sample of sheep: Belgium, Cyprus (where genotyping is carried out systematically in the breeding sheep herd), France, Germany, Greece, Italy, Latvia, the Netherlands, and Poland. Iceland, one of the seven non-EU countries that disclosed genotyping data, did so as well. 8.8% of the sheep population (with known NSP genotype) was susceptible to CS (NSP3, NSP3O, NSP4 and NSP5) in the subset of EU27 and the UK that carried out the activity in 2020, omitting data from Cyprus, which was lower than the 15.7 percent in 2019 and the 19.2 % in 2018. In the EU27 and the UK, following the changes in the legislation that entered intoforce in 2018, nine MS conducted the genotype to a random sample of sheep: Belgium, Cyprus

wheregenotyping is conducted systematically in the breeding sheep po This percentage stands at 44.4% in Greece and 27.3% in Italy, two of the countries with the highest case load in 2020, whereas it was between 12% and 30% in the remaining six MS ("The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021).

***Ovis aries aries* Nucleic acid concentration Result**

Blood samples were taken from those animals too. Invitrogen DNA isolation kit (Thermo Scientific, UK) was used for genomic DNA extraction. Then DNA concentrations have been measured using nanodrop (NanoDrop^{TM} 2000/2000c, Thermo, UK).

Table 9.

Blood sampling farms,age, Ovis aries aries race and gender

Region 1 : Dikmen			**Date: 11.06.2019**		
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
S.1.1	NC040437141	1	SAKIZ-Awassi hybrid	Ram	25.1
S.1.2	NC040437151	1	SAKIZ-Awassi hybrid	Ram	36.5
S.1.3	NC040437145	2	SAKIZ-Awassi hybrid	Ram	37.9
S.1.4	NC 032372561	2	SAKIZ-Awassi hybrid	Ram	44.3
S.1.5	NC 034810840	3	SAKIZ-Awassi hybrid	Ram	30
Region 2: Düzova			**Date: 17.07.2019**		

No	Tag no	Age	Race	Gender	Nucleic acid concentration (ng/ul)
S.2.1	RAM 1	11 Month	SAKIZ-Awassi hybrid	Ram	35.4
S.2.2	RAM 2	11 Month	SAKIZ-Awassi hybrid	Ram	35.5
S.2.3	RAM 3	11 Month	SAKIZ-Awassi hybrid	Ram	25.4
S.2.4	861897	3 Age	SAKIZ-Awassi hybrid	Sheep	25.5
S.2.5	861297	4 Age	SAKIZ-Awassi hybrid	Sheep	40.0
S.2.6	902467	3 Age	SAKIZ-Awassi hybrid	Sheep	39.2
S.2.7	902686	3 Age	SAKIZ-Awassi hybrid	Sheep	26.5
S.2.8	902521	2 Age	SAKIZ-Awassi hybrid	Sheep	33.6
S.2.9	861936	4 Age	SAKIZ-Awassi hybrid	Sheep	46.7
Region 4: Büyük Kaymaklı (Ercan)			**Date: 26.09.2019**		

No	Tag no	Age	Race	Gender	Nucleic acid concentration (ng/ul)
S.4.5	NC036903256		SAKIZ-Awassi hybrid	Ram	30
S.4.6	531026		SAKIZ-Awassi hybrid	Sheep	20.0
S.4.7	NUMARASIZ		SAKIZ-Awassi hybrid	Sheep	41.1
S.4.8	268131		SAKIZ-Awassi hybrid	Sheep	26.3

Region 5: Boğazköy			**Date : 20.08.2019**		
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
S.5.1	383816	2	SAKIZ	Ram	38.1
S.5.2	701056	2	SAKIZ-Awassi hybrid	Sheep	32.8
S.5.3	598529	1,5	SAKIZ-Awassi hybrid	Ram	27.7
S.5.4	90865092	3	Awassi	Sheep	24.6
S.5.5	328271	1	SAKIZ	Ram	20.0
S.5.6	O86986	2	Awassi	Sheep	22.9
S.5.7	679718	2	Awassi	Sheep	50.6
S.5.8	701206	3	SAKIZ	Sheep	29.3
S.5.9	NC036701034	Sakız	1,5	Sheep	29.6

S.5.10	701090	SAKIZ-Awassi hybrid	2	Sheep	24.6
S.5.11	KOÇ NUMARASIZ	Awassi	4	Ram	10.4

Region 6: Vadili			**Date: 27.09.2019**		
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
S.6.1	NC038943165	1	SAKIZ	Ram	37.5
S.6.2	943171	2	SAKIZ	Sheep	43.2
S.6.3	396931	2	SAKIZ	Sheep	36.3
S.6.4	O35747	2	SAKIZ	Sheep	24.0
S.6.5	794741	1,5	SAKIZ	Sheep	43.2
S.6.6	794785	2	SAKIZ	Sheep	36.3
S.6.7	O35871	4	SAKIZ	Sheep	87
S.6.8	943232	1,5	SAKIZ	Sheep	34.1
S.6.9	NC036035732	4	SAKIZ	Ram	52.8
S.6.10	O35493	3	SAKIZ	Sheep	30.6

Region 7: Düzova			**Date: 12.10.2019**		
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
S.7.1	NC038292498	2	Awassi	Sheep	40
S.7.2	NC032303046	3	Awassi	Sheep	42.1
S.7.3	NC028567520	4	Awassi	Sheep	301.2

PCR and Sequencing results in Sheep

Northern Cyprus Sheep 50 DNA samples has been sequenced to study the possible polymorphism that shows resistance or predisposition to scrapie. Three different genetic variations were detected in the targeted gene locations including R171Q, R171H, and R231T. The geographical distribution of genetic polymorphisms that were previously associated with scrapie diseases is shown in

Table 1. It was observed that the R171Q (0,42) haplotype was too high: Duzova, Vadili, and Boğazköy respectively. The second most detected single nucleotide polymorphism was R171H (0.10), which was detected in three different locations: Duzova, Dikmen and Büyük Kaymaklı respectively. R231T was the third most common (0.12) and only in detected Duzova, Dikmen and Büyük Kaymaklı. R171Q, R171H, and R231T polymorphisms were detected in Chios, Aww Asi and hybrids.

Table 10.

First PCR gel electrophoresis result.

	1
A	S.1.1
B	S.1.2
C	S.1.3
D	S.1.4
E	S.1.5
F	S.2.1
G	S.2.2
H	S.2.3

Figure 3.

First PCR gel electrophoresis result. The obtained PCR products of the PrP gene of sheep and goat samples.

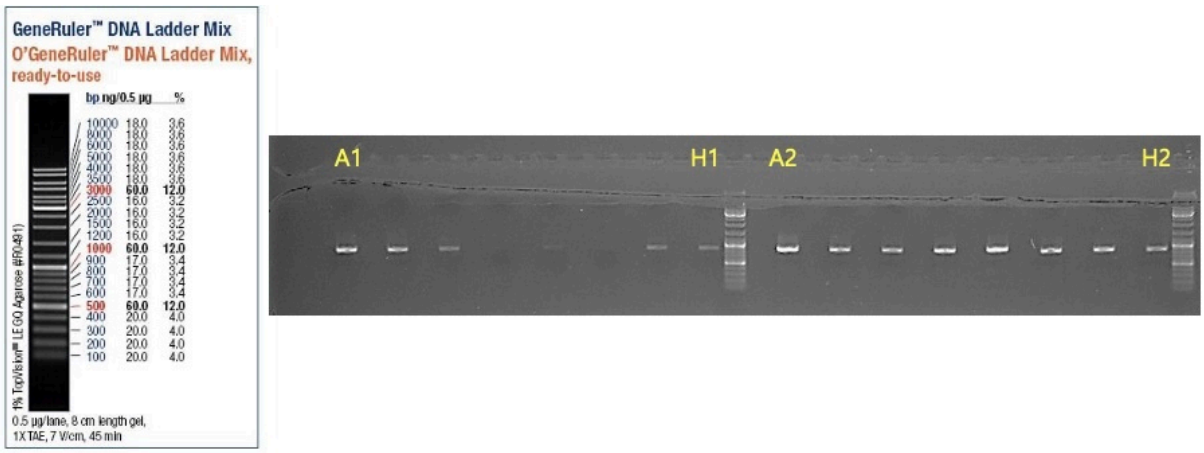


Table 11.

First PCR electro gel phoresis is result.

	1	2	3	4	5	6	7	8	9	10	11
A	S.2.4	S.4.7	S.5.7	S.6.4	S.7.1	S.8.7	G.2.13	G.3.4	G.3.12	G.3.20	G.7.5
B	S.2.5	S.4.8	S.5.8	S.6.5	S.7.2	S.8.8	G.2.14	G.3.5	G.3.13	G.4.1	G.7.6
C	S.2.6	S.5.1	S.5.9	S.6.6	S.7.3	G.2.7	G.2.15	G.3.6	G.3.14	G.4.2	G.7.7
D	S.2.7	S.5.2	S.5.10	S.6.7	S.8.1	G.2.8	G.2.16	G.3.7	G.3.15	G.4.3	G.7.8
E	S.2.8	S.5.3	S.5.11	S.6.8	S.8.2	G.2.9	G.2.17	G.3.8	G.3.16	G.7.1	
F	S.2.9	S.5.4	S.6.1	S.6.9	S.8.4	G.2.10	G.3.1	G.3.9	G.3.17	G.7.2	
G	S.4.5	S.5.5	S.6.2	S.6.10	S.8.5	G.2.11	G.3.2	G.3.10	G.3.18	G.7.3	
H	S.4.6	S.5.6	S.6.3	S.6.11	S.8.6	G.2.12	G.3.3	G.3.11	G.3.19	G.7.4	

Figure 4.

First PCR electro gel phoresis result. The obtained PCR products of the PrP gene of sheep and goat samples.

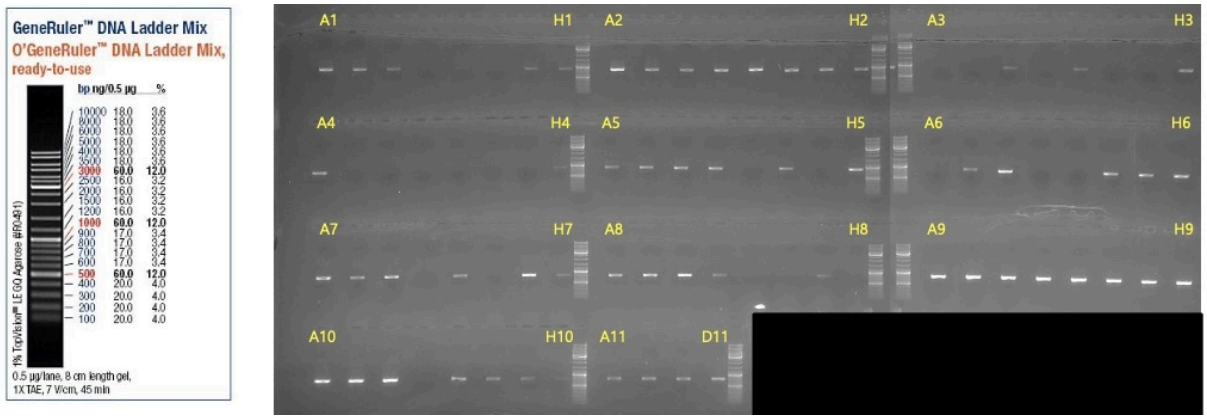


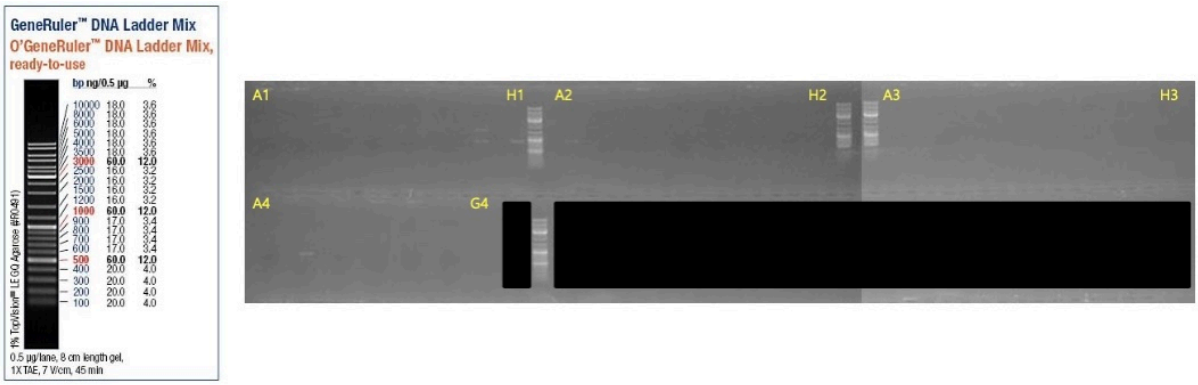
Table 12.

Second PCR electro gel phoresis is result.

	1	2	3	4
A	S.1.2	S.2.9	S.6.7	G.2.9
B	S.1.3	S.5.7	S.6.8	G.2.16
C	S.1.4	S.5.8	S.6.9	G.3.1
D	S.2.1	S.5.10	S.6.10	G.3.8
E	S.2.2	S.6.1	S.8.2	G.3.9
F	S.2.3	S.6.2	S.8.5	G.3.11
G	S.2.7	S.6.5	S.8.7	G.3.4
H	S.2.8	S.6.6	G.2.8	

Figure 5.

Secound PCR electro gel phoresis result. The PrP gene PCR products acquired from sheep and goat samples.



In the present study, five novel *PRNP* variants were detected in the Chios, Awassi, and hybrid sheep breeds. This study found R171Q, R171H, and novel *PRNP* variants R231T.

Table 13.

Estimated haplotype frequencies in the three populations studied: Chios, Awassi, and hyprid n: number of animals

Codon Position	171	231	Frequency (%) in		
			SAKIZ n=10	AWASSI n=15	HYBRID n=25
Hp1	R	R	70(7)	33.3(5)	24(6)
Hp2	Q	-	3(3)	53.3(8)	40(10)
Hp3	H	-		6.6(1)	16(4)
Hp4		T		6.6(1)	(20)5

Table 14.

Different genotype frequencies (percentages) were defined in their populations according to regions. n is the total number of animals.

Genotype	Frequency (%) in			
	SAKIZ n=10	**AWASSI n=15**	**HYBRID n=25**	**ALL**

	Common Homozygot	Heterezygot	Rare Homozygot	Common Homozygot	Heterezygot	Rare Homozygot	Common Homozygot	Heterezygot	Rare Homozygot	
R171Q	30 (3)		-	13.3(2)	13.3 (2)	26.6 (4)	-	16 (4)	24 (6)	42(21)
R171H	-	-	-	-	-	4 (1)	-	4 (1)	12(3)	10(5)
R231T	-	-	-	-	6.6(1)		-	16 (4)	4 (1)	12 (6)

Table 15.

PRNP allele frequencies (%) for sheep (ARQ, ARR, ARH, VRQ)

Haplotype	**Frequency (%) in**						
	SAKIZ n=10		**AWASSI n=15**		**HYBRID n=25**		**ALL**
	hetero	homo	hetero	homo	hetero	homo	
ARQ	30 (3)	-	13.3 (2)	40(6)	16 (4)	24 (6)	42(21)
ARR	-	-	-	-	-	-	-
ARH	-	-	-	4 (1)	4 (1)	12(3)	10(5)
VRQ	-	-	-	-	-	-	-

Table 16.

In their populations, different genotype frequencies (percentages) were identified according to region. The total number of animals is n.

Genotype	**Dikmen n=6**		**Düzova n=11**		**Büyük Kaymaklı =4**		**Boğaz n=11**		**Vadili n=10**		**Sütlüce n=8**	
	hetero	**homo**	**hetero**	**homo**	**hetero**	**homo**	**hetero**	**homo**	**hetero**	**homo**	**hetero**	**homo**
R171Q	-	-	46,46(5)	18,18(2)	50 (2)	25(1)	27,27 (3)	27,27 (3)	-	30 (3)	20 (2)	10 (1)
R171H	16.6 (1)	9.09 (1)	-	9.09 (1)	25(1)	-	-	-	-	-	-	10 (1)
R231T	16.6 (1)	9.09 (1)	18,18(2)	-	25(1)	-	-	-	-	-	10 (1)	-

Figure 5

ARQ and ARH haplotype frequency in sheep herds

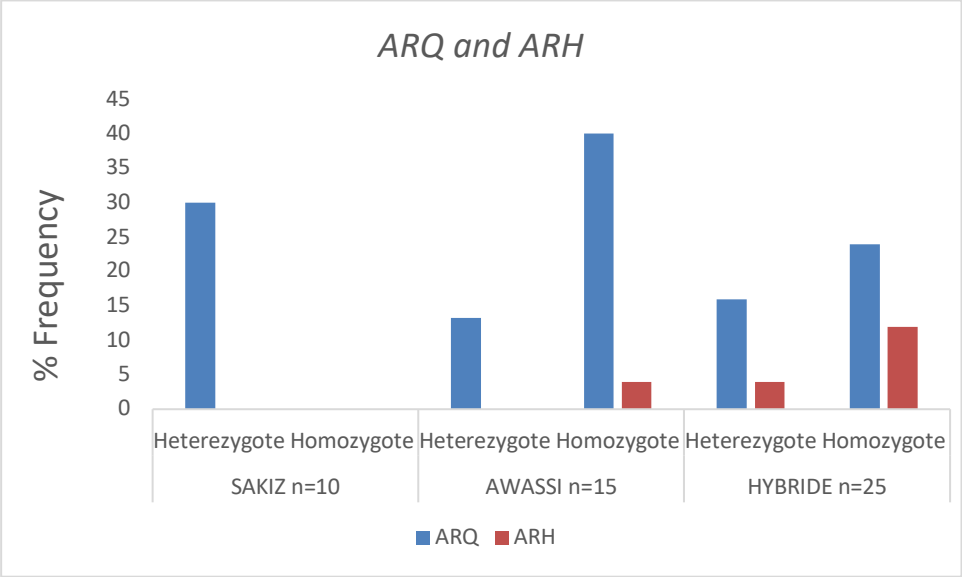
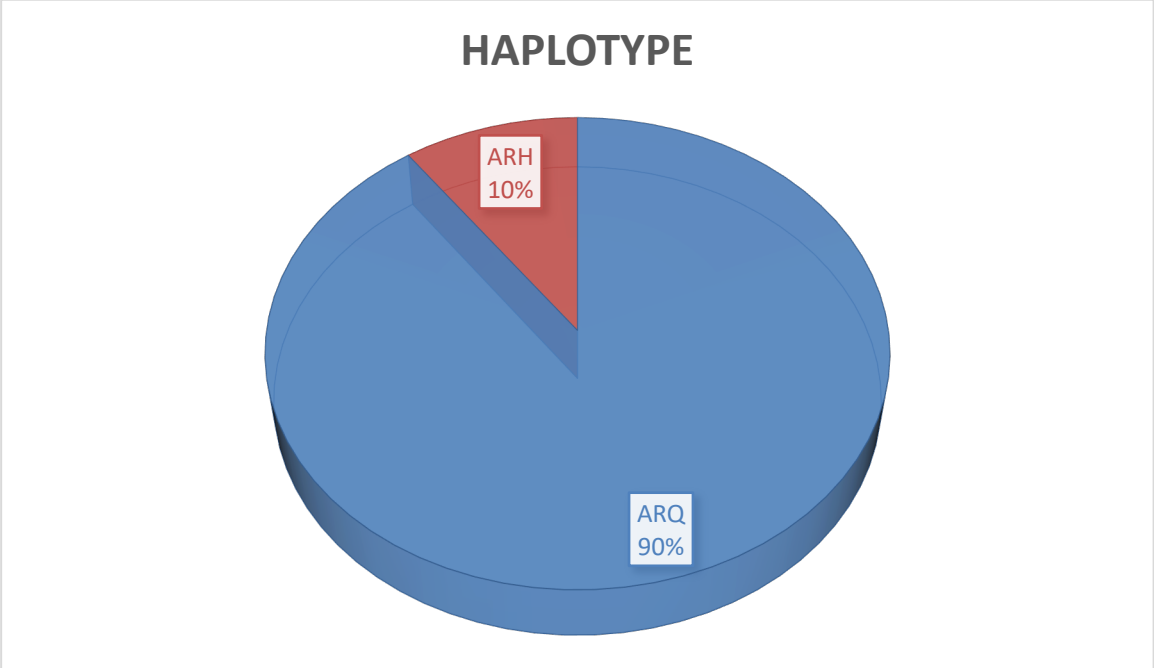


Figure 6.

Haplotype polymorphisms in the PRNP gene in Northern Cyprus sheep when included in wildtypes.



Discussion

Northern Cyprus sheep breeding ARQ genotype substantially to be seen. By European Union National Scrapie Plan ARQ/ARQ genotype is genetically little resistant (ARQ/ARQ may be scientifically reviewed). Nevertheless, the ARQ

genotype is reported to be susceptible. The frequency of sheep with the ARQ haplotype was determined to be 42% and ARH 10% in Awassi, Chios and crossbreeds in Northern Cyprus. No significant results were found with the statistical analysis (P=0,9998). Southern Cyprus has been using The National Scrapie Plan (NSP). Therefore, Northern Cyprus must be used NSP. Cyprus must be one scrapie plan and work together.

CHAPTER IV
Results of Goat PRNP Gene Sequencing

Introduction

The conversion of a normal cellular protein into an aberrant isoform causes transmissible spongiform encephalopathies (TSEs), often known as prion diseases, which are deadly neurodegenerative diseases (Akis et al., 2020). Many species, including humans, sheep, deer, and goats, are susceptible to TSEs because of variations in the prion protein (PRNP) gene (Zhou et al., 2008). The effects of PRNP polymorphisms at codons 136, 154, and 171 in sheep are particularly well documented. In sheep, the ARR allele is linked to resistance to classical scrapie, while the VRQ and ARQ alleles are linked to susceptibility (Akis et al., 2020). In recent decades, sheep breeding programs based on PRNP genotypes have been undertaken in European Union member countries to manage and eradicate scrapie. Due to a lack of data on the degree of resistance of scrapie-associated alleles, a comparable technique has not yet been used in goats (Ricci et al., 2017; Sacchi et al., 2018). In goats from Greece, Italy, and Cyprus, the PRNP codon 168 polymorphism has been found, and 168P homozygotes have been linked to scrapie susceptibility (S. K. Kim et al., 2019). In the previous decades, various polymorphisms have been examined for their association with scrapie. The 222K allele, which is particularly efficient against classical scrapie, and other polymorphisms 146D, 146S, 154H, and 211Q were related to a decreased risk of classical scrapie (Acutis et al. 2006; Barillet et al. 2009). (Acutis et al. 2006; Barillet et al. 2009). The European Food Security Authority (EFSA) presented a scientific opinion on the genetic resistance to TSEs in goats, based on a comprehensive evaluation of prior studies and suggested K222, D146, and S146 alleles as suitable resistance alleles for a breeding strategy In the previous decades, various polymorphisms have been examined for their association to scrapie. The 222K allele, which is particularly efficient against classical scrapie, and other polymorphisms 146D, 146S, 154H, and 211Q were related with a decreased risk towards classical scrapie. The European Food Security Authority (EFSA) presented a scientific opinion on the genetic resistance to TSEs in goats, based on a comprehensive evaluation of prior studies and suggested K222, D146 and S146 alleles as suitable resistance alleles for a breeding strategy (Ricci et al., 2017; Akis et al., 2020).

Data Collection and Study Design

A total of 50 goat blood samples were taken from four specific places in Northern Cyprus: Dikmen (n=2), Düzova (n=19), Akdeniz (n=20), and Büyük Kaymakl (n=3). Clinically healthy goats from the Cyprus Native Hair Goat (n = 20), Damascus (n = 16), and Damascus and Saanen hybrids (n = 14) breeds were sampled. The regions where the samples were taken, their ages and races are shown Table 17.

Blood sampling farms, age, Capra hircus race and gender

Region 1 : Dikmen		**Date: 11.06.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
G.1.1	NC040433723	**1**	**Damascus** hybrid	**Lodge**
G.1.2	NC0404337167	**3**	**Damascus** hybrid	**Lodge**
Region 2: Düzova		**Date:**		
No	**Ear no**	**Age**	**Race**	**Gender**
G.2.1	TEKE 1	10 Month	Damascus hybrid	Lodge
G.2.2	TEKE 2	10 Month	Damascus hybrid	Lodge
G.2.3	TEKE 3	9 Month	Damascus hybrid	Lodge
G.2.4	TEKE 4	11 Month	Damascus hybrid	Lodge
G.2.5	TEKE 5	10 Month	Damascus hybrid	Lodge
G.2.6	TEKE 6	10 Month	Damascus hybrid	Lodge
G.2.7	TEKE 7	9 Month	Damascus hybrid	Lodge
G.2.8	TEKE 8	10 Month	Damascus hybrid	Lodge
G.2.9	TEKE 9	11 Month	Damascus hybrid	Lodge
G.2.10	902473	6	Damascus	Goat
G.2.11	NO	6	Damascus	Goat
G.2.12	129707	2	Damascus	Goat

G.2.13	824825	4	Damascus	Goat
G.2.14	TEKE 10	1,5	Damascus	Lodge
G.2.15	409635	3	Damascus	Goat
G.2.16	902378	4	Damascus	Goat
G.2.17	O1092	3	Damascus	Goat

Region 3: Akdeniz Native Cyprus Goat		**Date: 17.07.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
G.3.1	403897	-	Native Cyprus Goat	Goat
G.3.2	213861	-	Native Cyprus Goat	Goat
G.3.3	213716	-	Native Cyprus Goat	Goat
G.3.4	157943	-	Native Cyprus Goat	Goat
G.3.5	NC04015847	-	Native Cyprus Goat	Goat
G.3.6	157254	-	Native Cyprus Goat	Goat
G.3.7	6209	-	Native Cyprus Goat	Goat
G.3.8	407626	-	Native Cyprus Goat	Goat
G.3.9	629516	-	Native Cyprus Goat	Goat
G.3.10	402901	-	Native Cyprus Goat	Goat
G.3.11	407878	-	Native Cyprus Goat	Goat
G.3.12	NC02283958	-	Native Cyprus Goat	Goat

G.3.13	NC030406952	-	Native Goat	Cyprus	Goat
G.3.14	216529	-	Native Goat	Cyprus	Goat
G.3.15	158053	-	Native Goat	Cyprus	Goat
G.3.16	213627	-	Native Goat	Cyprus	Goat
G.3.17	627974	-	Native Goat	Cyprus	Goat
G.3.18	630205	-	Native Goat	Cyprus	Goat
G.3.19	129643	-	Native Goat	Cyprus	Goat
G.3.20	407860	-	Native Goat	Cyprus	Goat

Region 4 : Büyük Kaymaklı		**Date: 26.09.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
G.4.1	531053	**3**	Damascus	Goat
G.4.2	807740	**4**	Damascus	Goat
G.4.3	459287	**4**	Damascus	Goat

Region 7: Düzova		**Date: 12.10.2019**		
No	**Ear no**	**Age**	**Race**	**Gender**
G.7.1	NC030858280	4	Damascus hybrid	Goat
G.7.2	NO	4	Damascus	Goat
G.7.3	NC038292179		Damascus	Goat
G.7.4	NC036806576	5	Damascus	Goat
G.7.5	NC0269939540		Damascus	Goat
G.7.6	NC026860393	4	Damascus	Goat
G.7.7	NC026759745		Damascus	Goat
G.7.8	NC0269385406	6	Damascus	Goat
G.7.1	NC030858280	4	Damascus hybrid	Goat
G.7.8	NC0269385406	4	Damascus	Goat

Capra Hircus Race Nucleic Acid Concentration Result

Blood was obtained from the animals as well. Genomic DNA was extracted using an Invitrogen DNA isolation kit (Thermo Scientific, UK). The quantities of DNA were then determined using nanodrop (Thermo, UK, NanoDropTM 2000/2000c). Table 18.

Blood sampling farms,age, Capra hircus race and gender.

Region 1 : Dikmen		**Date: 11.06.2019**			
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
G.1.1	NC040433723	1	Damascus hybrid	Lodge	20.9
G.1.2	NC0404337167	3	Damascus hybrid	Lodge	34.1
Region 2: Düzova		**Date:**			
No	**Tag no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration (ng/ul)**
G.2.1	TEKE 1	10 Month	Damascus hybrid	Lodge	22.4
G.2.2	TEKE 2	10 Month	Damascus hybrid	Lodge	28.5
G.2.3	TEKE 3	9 Month	Damascus hybrid	Lodge	52.2
G.2.4	TEKE 4	11 Month	Damascus hybrid	Lodge	59.3
G.2.5	TEKE 5	10 Month	Damascus hybrid	Lodge	32.3
G.2.6	TEKE 6	10 Month	Damascus hybrid	Lodge	40.5
G.2.7	TEKE 7	9 Month	Damascus hybrid	Lodge	35.0

G.2.8	TEKE 8	10 Month	Damascus hybrid	Lodge	21.4
G.2.9	TEKE 9	11 Month	Damascus hybrid	Lodge	21.6
G.2.10	902473	6	Damascus	Goat	20.6
G.2.11	NO	6	Damascus	Goat	36.7
G.2.12	129707	2	Damascus	Goat	35.6
G.2.13	824825	4	Damascus	Goat	37.3
G.2.14	TEKE 10	1,5	Damascus	Lodge	33.4
G.2.15	409635	3	Damascus	Goat	24.1
G.2.16	902378	4	Damascus	Goat	23.7
G.2.17	O1092	3	Damascus	Goat	31.2

Region 3: Akdeniz Native Cyprus Goat	**Date: 17.07.2019**				
No	**Ear no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration**
G.3.1	403897		Native Cyprus Goat	Goat	50 ng / µl
G.3.2	213861		Native Cyprus Goat	Goat	29.1
G.3.3	213716		Native Cyprus Goat	Goat	55.5
G.3.4	157943		Native Cyprus Goat	Goat	24.8
G.3.5	NC04015847		Native Cyprus Goat	Goat	46.9
G.3.6	157254		Native Cyprus Goat	Goat	27.7
G.3.7	6209		Native Cyprus Goat	Goat	24.0
G.3.8	407626		Native Cyprus Goat	Goat	85

G.3.9	629516	Native Cyprus Goat	Goat	30.3
G.3.10	402901	Native Cyprus Goat	Goat	46.4
G.3.11	407878	Native Cyprus Goat	Goat	25
G.3.12	NC02283958	Native Cyprus Goat	Goat	30.1
G.3.13	NC030406952	Native Cyprus Goat	Goat	42.4
G.3.14	216529	Native Cyprus Goat	Goat	32.8
G.3.15	158053	Native Cyprus Goat	Goat	38.6
G.3.16	213627	Native Cyprus Goat	Goat	62.4
G.3.17	627974	Native Cyprus Goat	Goat	63.3
G.3.18	630205	Native Cyprus Goat	Goat	54.3
G.3.19	129643	Native Cyprus Goat	Goat	50.8
G.3.20	407860	Native Cyprus Goat	Goat	48.4

Region 4 : Büyük Kaymaklı		**Date: 26.09.2019**			
No	**Ear no**	**Age**	**Race**	**Gender**	**Nucleic acid concentration**
G.4.1	531053	**3**	Damascus	Goat	64.3
G.4.2	807740	**4**	Damascus	Goat	47.4
G.4.3	459287	**4**	Damascus	Goat	22.3

Region 7: Düzova	**Date: 12.10.2019**

No	Ear no	Age	Race	Gender	Nucleic acid concentration
G.7.1	NC030858280	4	Damascus hybrid	Goat	47.6
G.7.2	NO	4	Damascus	Goat	26.
G.7.3	NC038292179		Damascus	Goat	23.0
G.7.4	NC036806576	5	Damascus	Goat	20.2
G.7.5	NC0269939540		Damascus	Goat	47
G.7.6	NC026860393	4	Damascus	Goat	38.8
G.7.7	NC026759745		Damascus	Goat	23
G.7.8	NC0269385406	6	Damascus	Goat	
G.7.9	NC030858280	4	Damascus hybrid	Goat	
G.7.10	NC0269385406	4	Damascus	Goat	

PCR and Sequencing results

The investigated polymorphism resulted in a genotypable P42= (CCG>CCA), Gly127ser (GGC>AGC), Ser138= AGT>AGC, Asn146Ser (AAT>GAT), Arg154His (CGT>CAT), Gln172Arg (CAG>CGG), Gln172His (CAG>CAT), Gln172Arg (CAG>CGT), Val179Val (GTG>GTT), Val187Val (CAG>CAT).

Table 19.

First PCR gel electrophoresis is result. Samples marked in yellow did not work.

	2
A	G.1.1
B	G.1.2
C	G.2.1
D	G.2.2
E	G.2.3
F	G.2.4
G	G.2.5
H	G.2.6

Figure 7

First PCR electro gel phoresis result. The obtained PCR products of the PrP gene of sheep and goat samples.

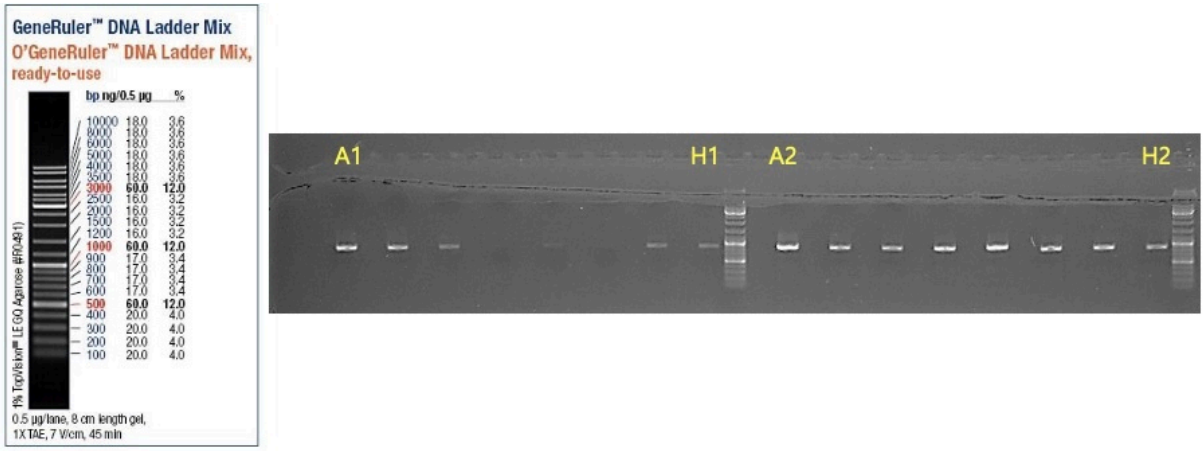


Table 20.

First PCR electro gel phoresis is result.

	1	2	3	4	5	6	7	8	9	10	11
A	S.2.4	S.4.7	S.5.7	S.6.4	S.7.1	S.8.7	G.2.13	G.3.4	G.3.12	G.3.20	G.7.5
B	S.2.5	S.4.8	S.5.8	S.6.5	S.7.2	S.8.8	G.2.14	G.3.5	G.3.13	G.4.1	G.7.6
C	S.2.6	S.5.1	S.5.9	S.6.6	S.7.3	G.2.7	G.2.15	G.3.6	G.3.14	G.4.2	G.7.7
D	S.2.7	S.5.2	S.5.10	S.6.7	S.8.1	G.2.8	G.2.16	G.3.7	G.3.15	G.4.3	G.7.8
E	S.2.8	S.5.3	S.5.11	S.6.8	S.8.2	G.2.9	G.2.17	G.3.8	G.3.16	G.7.1	
F	S.2.9	S.5.4	S.6.1	S.6.9	S.8.4	G.2.10	G.3.1	G.3.9	G.3.17	G.7.2	
G	S.4.5	S.5.5	S.6.2	S.6.10	S.8.5	G.2.11	G.3.2	G.3.10	G.3.18	G.7.3	
H	S.4.6	S.5.6	S.6.3	S.6.11	S.8.6	G.2.12	G.3.3	G.3.11	G.3.19	G.7.4	

Figure 20.

First PCR electro gel phoresis result. The obtained PCR products of the PrP gene of sheep and goat samples.

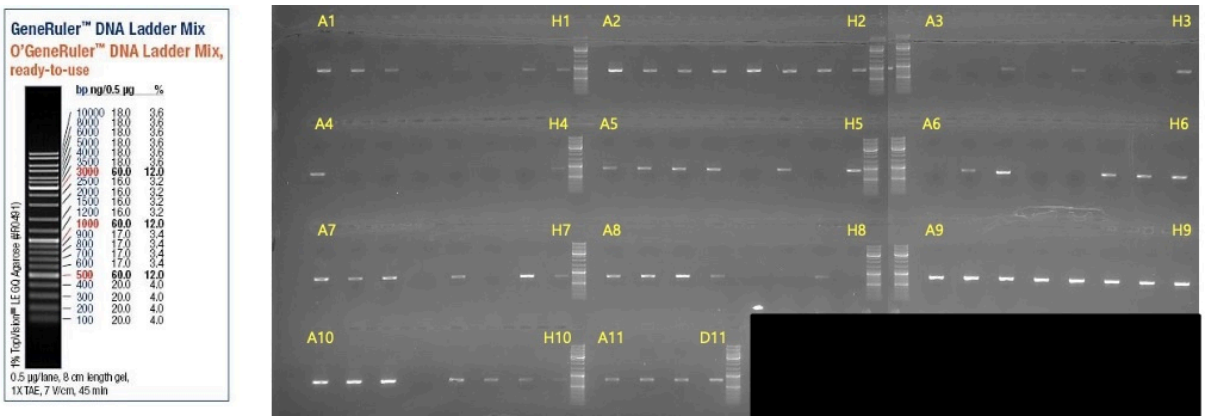


Table 21.

Second PCR electro gel phoresis is result. Samples marked in yellow did not work.

	1	2	3	4
A	S.1.2	S.2.9	S.6.7	G.2.9
B	S.1.3	S.5.7	S.6.8	G.2.16
C	S.1.4	S.5.8	S.6.9	G.3.1
D	S.2.1	S.5.10	S.6.10	G.3.8
E	S.2.2	S.6.1	S.8.2	G.3.9
F	S.2.3	S.6.2	S.8.5	G.3.11
G	S.2.7	S.6.5	S.8.7	G.3.4
H	S.2.8	S.6.6	G.2.8	

Figure 8

Secound PCR electro gel phoresis result. The PrP gene PCR products acquired from sheep and goat samples.

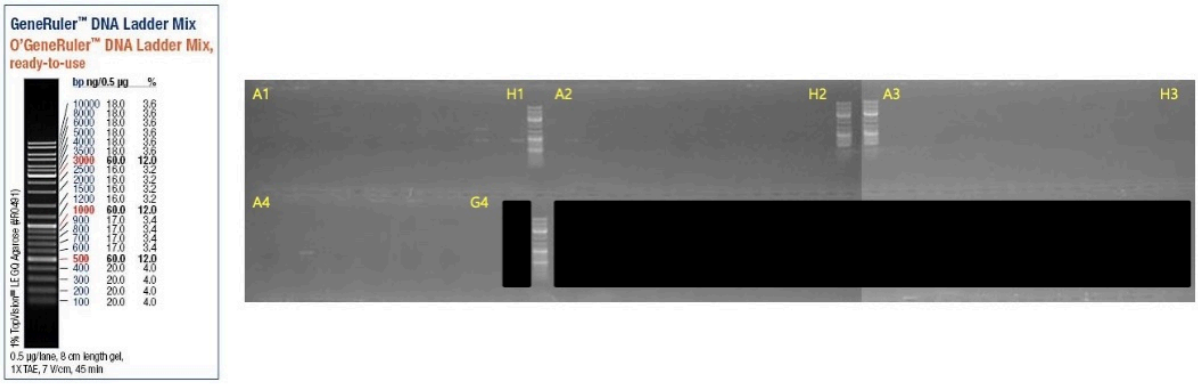


Table 22.

Different genotype frequencies (percentages) were defined in their populations according to regions. n is the total number of animals.

					Dikmen (N=2)		Düzova (N=25)		Akdeniz (cyprus native goat) (N=20)		Büyük Kaymaklı (n=3)		All (N=50)
p.(Pro42Pro)	**p.(Gly127Gly)**	**p.(Asn146Asn)**	**p.(Arg154Arg)**	**n**	**Genotypes Frequency**	**n**	**Genotypes Frequency**	**n**	**Genotypes Frequency**	**n**	**Genotypes Frequency**	
p.(Pro42Pro)	-	-	-	-	-	1	0,04	6	0,3	1	0,04(1)	0,16
-	p.(Gly127Ser)	-	-	-	-	1	0,04	-	-	-	-	0,02
	-	p.(Asn146Ser)	-	-	-	4	0,16	-	-	-	-	0,08
			p.(Arg154His)	-	-	4	0,16	2	0,2	-	-	0,12

Table 23.

Different genotype frequencies (percentages) identified in native goat (NGP), Damascus and hybrid goat (CGP) populations. n is the total number of animals.

Polymorhisms				Damascus (N=16)		Akdeniz (Cyprus Native hair Goat) (N=20)		Hybrid (N=14)		All (N=50)
p.(P42P)	**p.(G127G)**	**p.(N146N)**	**p.(R154R)**	**N**	**%**	**N**	**%**	**N**	**%**	**%**
p.(P42=)	-	-	-	1	6,25	6	30	1	14,29	16
-	p.(G127S)	-	-	1	6,25	-	-	1	14,29	4
	-	p. (N146S)	-	3	18,75	-	-	4	28,57	14
			p.(R154H)	-	-	2	10	2	14,29	8

Haplotype polymorphisms in the *PRNP* gene in Northern Cyprus goats when included in wildtypes. Sequencing analysis was performed on SnapGene software (GSL Biotech LLC, USA). An ANOVA tests non-parametric Friedman test was applied to check each locus

Discussion

p. (Asn146Ser) and p.(Arg154His) in Northern Cyprus scrapie eradication for goats need to focus on two results. That two genotypes can provide resistance to scrapie. Cyprus Native Hair Goats have a 154H genotype of 10% that means can use a goat specie eradication plan. Damascus (18,75%) and hybrid (28,57) goats have 146S genotype. We can use this resistant genotype for the eradication program.

CHAPTER V
Discussions

Introduction

TSEs are protein misfolding illnesses that are uncommon in that they may be passed from human or animal. Some TSEs, for example, can be passed from person to person through body fluids (Zhang & Zhang, 2013; Teferedegn et al., 2020; Chong et al., 2021). Prion diseases represent a family of transmissible slowly developing and always lethal neurodegenerative diseases affecting humans and animals (Torricelli et al., 2021). There are no gross neuropathological lesions in TSEs, but there are histological lesions that are common: bilateral and symmetrical widespread neuropil and/or neuronal vacuolation (spongiform appearance), synaptic changes, neuronal loss, gliosis, a variable degree and type of PrP^{Sc} accumulation, and occasionally amyloid plaques (Budka, 2003; Orge et al., 2021; *Veterinary Neuropathology: Essentials of Theory and Practice - Robert Higgins, Anna Oevermann, Marc Vandevelde - Google Books*, n.d.).

Scrapie in sheep and goats is a prion disease caused by a transmissible spongiform encephalopathy (TSE) that was originally diagnosed approximately 300 years ago in the United Kingdom and other Western European nations. Scrapie has been recorded all over the world since then, with the noteworthy exception of Australia and New Zealand. Scrapie may spread quickly throughout a flock. It results in yearly economic losses of up to $20 million in the United States owing to decreased productivity, lost exports, and increased carcass disposal costs (Greenlee, 2019a).

Association of *PRNP* Gene with Scrapie in Sheep

Classical scrapie was the first TSE to be discovered in sheep in the United Kingdom, more over 270 years ago (UK). The first natural case of caprine scrapie was not documented until 1942, and, aside from numerous cattle outbreaks, natural BSE infection was not identified in a single British goat until 2005. Later, a Scottish goat infected with BSE in 1990 was identified in retroactive examinations conducted

in the United Kingdom. It is a fatal, infectious illness with a long incubation period, mainly affecting animals between the ages of 2 and 5, and infected animals can live between 1 and 6 months after clinical indications develop (Acín et al., 2021).

The European Union and the United Kingdom (UK) reported a total of 688 cases of scrapie in sheep in the year 2020, which is 309 fewer than in the previous year, 2019. Two of the four countries that are not part of the EU reported a total of 65 cases of scrapie in sheep (Iceland and Norway). Classical scrapie (CS) was found in seven EU countries and one non-EU country, including Bulgaria, Cyprus, Greece, Italy, Portugal, Romania, and Spain; atypical scrapie (AS) was found in Belgium, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Poland, Portugal, Romania, Slovakia, Spain, and Sweden, in addition to the United Kingdom (UK) and another non-EU country; both types of scrapie were found in Iceland. Classical scrapie was found in (Norway). In addition, twelve suspect cases of scrapie in sheep were registered in Italy; however, these cases were not counted toward the overall number of scrapie cases reported in the country (“The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020,” 2021).

The *PRNP* genotype for codons 136, 154, and 171 has a clear effect on the susceptibility to classical scrapie. Previous research has linked three polymorphic codons in sheep *PRNP* to scrapie resistance/susceptibility (136 A (Alanine)/V (Valine), 154 R (Arginine)/H (Histidine), and 171 Q (Glutamine)/R/H). Alanine, arginine, and arginine at codons 136, 154, and 171, respectively, are linked to scrapie resistance in classical forms. PrP variations (VRQ or ARQ) are, on the other hand, linked to vulnerability (Greenlee, 2019b; Alarcon et al., 2021; Marín-Moreno et al., 2021).

Some scrapie strains target the VRQ gene, whereas others prefer the wild-type ARQ allele, which is probably right. In natural scrapie, however, it appears that the VRQ allele is frequently targeted if it is present in sufficient numbers (Tranulis, 2002). ARQ genotype was dominant in both healthy and scrapie-infected sheep breeds sometimes (Teferedegn, Yaman, & Un, 2020). Great Britain - The National Scrapie Plan is considered to be ARQ/ARQ, ARH/ARQ and ARH/ARH R3 when looking at the average scoring according to risk groups and genotypes desirable or

undesirable for selective breeding against Scrapie. (R3= genetically less resistant to scrapie) (Betmezoğlu & Arsoy, 2019; Yaman et al., 2020). The frequency of sheep with the ARQ haplotype was determined to be 42% and ARH 10% in Northern Cyprus. The emergence of genetically resistant ARQ sheep suggests that classical scrapie might be eradicated by genetic selection without the need of ARR rams (Heaton et al., 2010).

In sheep, the EU27 and the UK recorded 688 scrapie incidents in 2020, 309 less than in 2019. In all, 65 instances of scrapie in sheep were reported by two of the four non-EU countries that tested sheep (Iceland and Norway) ("The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021). Cyprus herds have no differentiation between HGMF and CF, and all flocks are breaded as a single-tier with doing for to the BP-CS ("Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats," 2014a).

Although there is no official eradication effort in Northern Cyprus, an eradication program was implemented in 2003, which resulted in the slaughter of roughly 3000 sheep and the payment of compensation to the farmers. Following that, due to economic challenges, this could not be sustained. Northern Cyprus farmers have purchased rams with ARR/ARR genotypes from Southern Cyprus farmers on an unofficial basis. Scrapie illness is becoming less common in Northern Cyprus as a result of this issue (Betmezoğlu & Arsoy, 2019). Northern Cyprus still does not have an eradication program. Looking at the results of this study, farms in the north of Cyprus are of risk to Scrapie disease.

In the population under research, nonsynonymous and synonymous substitutions such as G127G, S138S, R231R, L237L, G126A, 126GA, 127GV, 127GA, 142IT, N146S, N146NS were also discovered (Vaccari et al., 2007; Kdidi et al., 2014; Teferedegn et al., 2020). In this study, R231T new polymorphism was also identified in the population. In Awassi and Hybrids, 12 % of R231T polymorphism was discovered.

In Europe Union has been using the National Scrapie Plan (NSP). Therefore Southern Cyprus has been implementing that plan. Every year has been doing a genotype test for scrapie diseases that animals are resistant to genotype reclamation. Northern Cyprus for sheep breeding hasn't got any scrapie eradication program yet.

For this reason, animals' and humans' health have at risk; the government has to do a Scrapie plan.

Association of *PRNP* Gene with Scrapie in Goat

In goats, a large number of polymorphisms have been identified as protective or vulnerable alleles (Teferedegn, Yaman, & Ün, 2020). In 2020, the European Union (27 countries) and the United Kingdom tested a total of 453,194 small ruminants: 332,579 sheep (a reduction of 1.6% from 2019) and 120,614 goats (a 16 percent decrease). In addition, 26,053 sheep and 712 goats were examined by three of the seven non-Europian Union countries, namely Iceland, North Macedonia, Norway, and Serbia. In the European Union and the United Kingdom, 328 scrapie cases were documented in goats: nine Asymptomatic Scrapie cases and 319 Classical Scrapie cases (97.3 with Cyprus accounting for 74% of these). Compared to 2019, when 379 cases of Classical Scrapie were reported, there was a decline of 15.8 percent (60) due mostly to the situation in Cyprus, which has steadily improved over the past seven years. Four Member States (Bulgaria, Cyprus, Romania, and Greece) and the United Kingdom reported cases of Classical Scrapie, while three Member States (Denmark, France, and Portugal) reported cases of Asymptomatic Scrapie. Italy and Spain reported both Classical and asymptomatic forms of the disease. The other three non-European Union countries (Iceland, Serbia, and Norway) with tested goats did not record any occurrences of scrapie (“The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020,” 2021).

In goats, Iceland accounted for 18.6% of all cases recorded in the European Union (27 countries) and the United Kingdom in 2020, up from 8.7% in 2019, with a greater proportion of Asymptomatic Scrapie cases (100%) than Classical Scrapie cases (16.3 %)(“The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020,” 2021). In both species, Classical Scrapie continues to be the most often reported kind of scrapie in the European Union.

The most common replacements in the world are G127S, M142I, H143R, N146S, R154H, Q222K, and S240P (Ricci et al., 2017b). R139S in Algeria9, A116V

in Tanzania 10, G134E and Q163P in Turkey 11, and G127A and T193I in Ethiopia were all unique replacements found in their respective areas of research at the time of the publication. Experiments have shown that alleles like M142, D145, S146, H154, Q211, and K222 can either prolong the incubation time or boost resistance to scrapie (Wilfred Goldmann, 2008; W. Goldmann et al., 2016; Ricci et al., 2017; Kim & Jeong, 2018; Salvesen et al., 2020; Teferedegn, Yaman, & Ün, 2020).

Investigations have shown that specific codons within the goat *PRNP* gene are strongly connected, such as codons 42, 138, 179 and 240 (Acutis et al., 2008; Pier Luigi Acutis et al., 2006; Babar et al., 2009; Lan et al., 2012; Kdidi et al., 2021;). In the Marker assisted selection (MAS) breeding program, genetic variability at different codons of the coding region inside the *PRNP* gene is becoming a focus of interest investigating the relationship between genetic variation and scrapie infections and production attributes (Lan et al., 2012). Theoretically, there was codon bias in the P42P and its strong associated codon S138S of the goat *PRNP* gene (Colussi et al., 2008; Lan et al., 2012). These synonymous SNPs within the goat *PRNP* gene may control the expression of genes linked to production attributes, based on codon bias and a significant correlation between codons 42 and 138 and new polymorphisms at codons 172 and 179 have been discovered.

In this study p.(Gly127Ser), p. (Asn146Ser) and p.(Arg154His) haplotypes were seen. The study's most important finding was 146 codons. Due to the lack of or very low frequencies of alleles D146 and S146 across Europe, with the exception of Cyprus, no data from field investigations on genotypes at codon 146 resistance to European scrapie strains have been released to date (Nonno et al., 2020; Gelasakis et al., 2021). As a result, the European Food Safety Authority (EFSA) decided that additional epidemiological research was needed in the instance of codon 146 (“The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020,” 2021). In this study, 4% of the participants discovered the NS haplotype. However, the NN haplotype was also found at the same rate.

In our investigation, allele 154H was also linked to natural scrapie resistance. In Greek and Cypriot goats, where this gene appears to be somewhat protective against scrapie, a similar effect has previously been observed (Belt et al., 1995; Papasavva-

Stylianou et al., 2007b, 2011). Histidine at codon 154, according to the findings, is also strongly linked to the condition in goats, (Colussi et al., 2008; Samia Kdidi et al., 2021). The haplotype 154H was found at a rate of 16% at two different farms in the Düzova region and 2% in the Cyprus native goats in the Akdeniz region. The 154H polymorphism was discovered in 12% of the participants in this investigation. Considering the results obtained, it has been determined that a strict improvement policy is needed throughout Cyprus.

PRNP gene S146 and D146 polymorphisms naturally confer considerable protection to the illness, according to studies performed in Cyprus. HGMF (High Genetic Merit Folks) or BP-SC (Breeding Program Classical Scrapie) standards are used to breed herds all over the world. However, only BP-SC standards are used in Cyprus. However, only BP-SC standards are used in Cyprus; A special case is that all farms in Cyprus are included in the BP-SC. ("Scientific Opinion on the Scrapie Situation in the EU after 10 Years of Monitoring and Control in Sheep and Goats," 2014b). Goats in 2020; EU27 and UK, a total of 328 Scrapie cases were documented, with 9 AS and 319 CS (74 percent of Classical Scrapie cases occurring in Cyprus) ("The European Union Summary Report on Surveillance for the Presence of Transmissible Spongiform Encephalopathies (TSE) in 2020," 2021).

In European Union, the National Scrapie Plan has been implemented (NSP). Southern Cyprus has therefore been executing this idea. Each year, a genotyping test for scrapie diseases that animals are resistant to has been conducted. Northern Cyprus has no scrapie eradication program for goat breeding as of yet. Therefore, both animal and human health are at risk; the government must implement a Scrapie plan.

CHAPTER VI
Conclusion

Sheep

As a result, finding geographically relevant scrapie resistance genes in sheeps remains difficult. Based on the selection of animals having the ARR haplotype, the findings of this case-control study might surely give a tool for minimizing scrapie in sheep in Northern Cyprus. To avoid a Scrapie breakout in Northern Cyprus, more stringent precautions should be implemented. Clinical Veterinary Genetics should receive increased attention across the island.

Goat

Therefore, locating geographically relevant genes for scrapie resistance breeding in goats remains a difficulty. Based on the selection of animals carrying the amino acids aspartic acid (D) and serine (S) at this position, the results of this case-control study demonstrate that polymorphisms at codon 146 could serve as a tool for preventing scrapie in goats in Northern Cyprus, if animals carrying these amino acids are prioritized. Additional research into the effects of alleles 146D, 146S, and 154H on animals carrying these alleles, as well as the effects of these alleles on various TSE agents, could provide accurate and reliable information on the effectiveness of breeding for these alleles as a means of controlling and eliminating classical scrapie in goats. Additional research into the effects of alleles 146D, 146S, and 154H on animals carrying these alleles, as well as the effects of these alleles on various TSE agents, could provide accurate and reliable information on the effectiveness of breeding for these alleles as a means of controlling and eliminating classical scrapie in goats. To prevent an outbreak of Scrapie in Cyprus, additional precautions are required. Clinical Veterinary Genetics should receive a higher priority throughout the entire island. Budgets for breeding initiatives should be increased.

Future Marks

Northern Cyprus can use as a precursor that study for Scrapie eradication program. Additionally, the creation of variant-specific will enable us to further analyze the effect of variations detected in our patients and identify genotype associations and the Near East University Veterinary Faculty Molecular Biology and Genetic can laboratory use these tests. Additionally will be a pioneering work for our future work and can do more detailed studies in the coming years.

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Appendices

Appendix A

Ethical Approval Document

YAKIN DOĞU ÜNİVERSİTESİ
HAYVAN DENEYLERİ YEREL ETİK KURULU
ARAŞTIRMA PROJESİ DEĞERLENDİRME RAPORU

Toplantı Tarihi : **22/05/2019**
Toplantı No : **2019/05**
Proje Başvuru No : **75**

Yakın Doğu Üniversitesi, Veteriner Hekimliği Fakültesi'nden, Sorumlu Araştırmacı Doç. Dr. Dilek ARSOY tarafından hazırlanan "Kuzey Kıbrıs'ta sağlıklı keçi ve koyunlarda prion protein geni (PrP) polimorfizmi ve filogenetik çalışmalar." başlıklı araştırma, Kurulumuz tarafından etik olarak uygun bulunmuştur.

Prof. Dr. Emine KOÇ (BAŞKAN)

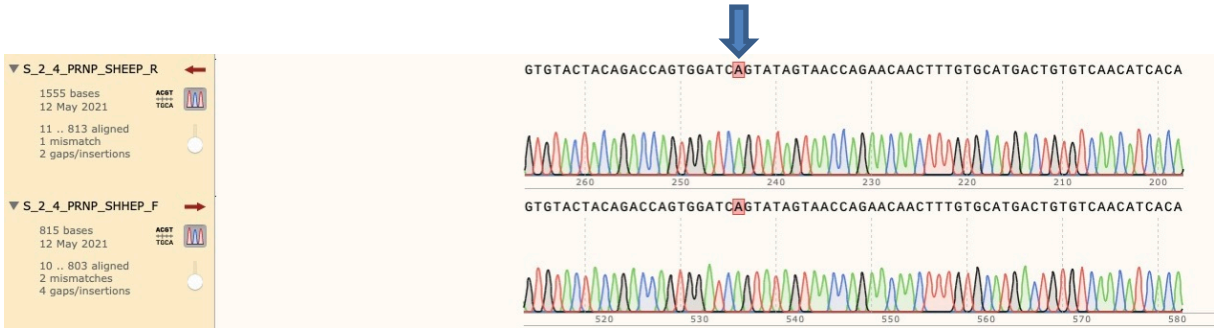
1. Prof. Dr. Tamer YILMAZ (ÜYE)
2. Prof. Dr. Vedat SAĞMANLIGİL (ÜYE)
3. Doç. Dr. Dilek ARSOY (ÜYE)
4. Doç. Dr. Bilgen BAŞGUT (ÜYE)
5. Doç. Dr. Serdar SUSEVER (ÜYE)
6. Yrd. Doç. Dr. Savaş Volkan GENÇ (ÜYE)
7. Vet. Hek. Umut SAYILI (ÜYE)
8. Avukat Burak NOLAN (ÜYE)
9. Vet. Hek. Meliha TEMİZEL (ÜYE)

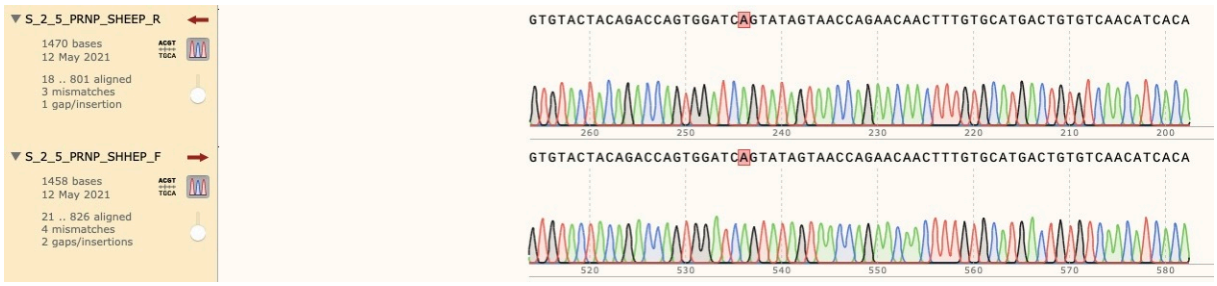
Appendix B

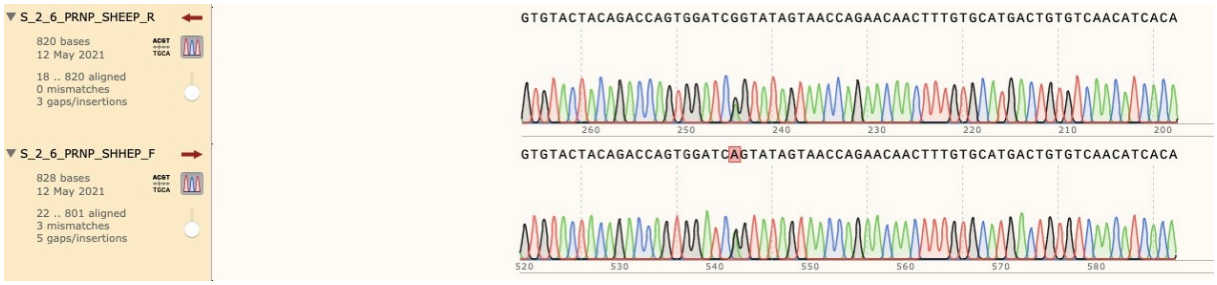
Signed Similarity Report

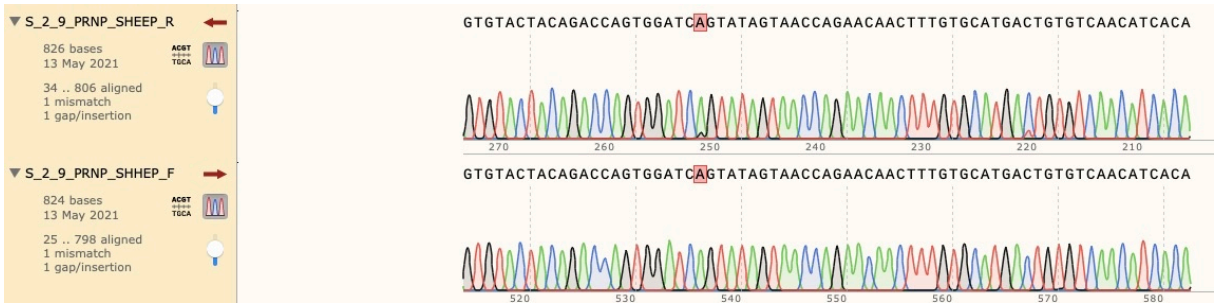
Sequence Reports
Sheep

R171Q Genotype Sequencing Results








S_2_9_PRNP_SHEEP_R
826 bases
13 May 2021
34 .. 806 aligned
1 mismatch
1 gap/insertion
S_2_9_PRNP_SHHEP_F
824 bases
13 May 2021
25 .. 798 aligned
1 mismatch
1 gap/insertion
GTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACAACTTTGTGCATGACTGTGTCAACATCACA
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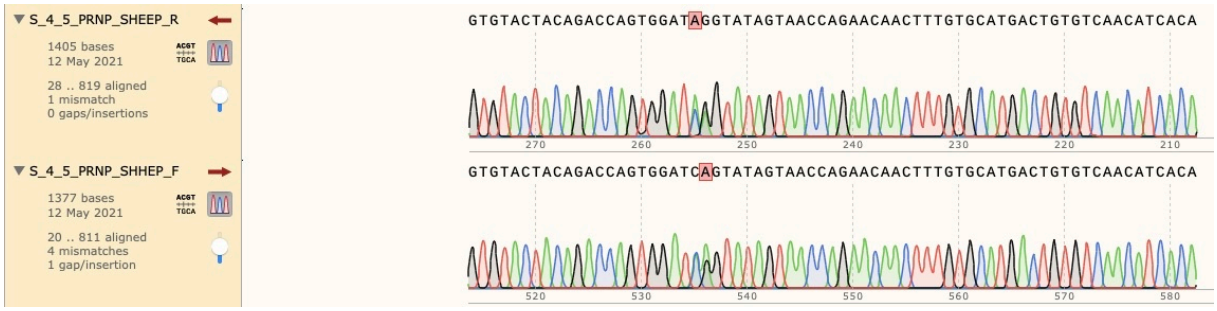
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1405 bases
12 May 2021
28 .. 819 aligned
1 mismatch
0 gaps/insertions
S_4_5_PRNP_SHHEP_F
1377 bases
12 May 2021
20 .. 811 aligned
4 mismatches
1 gap/insertion
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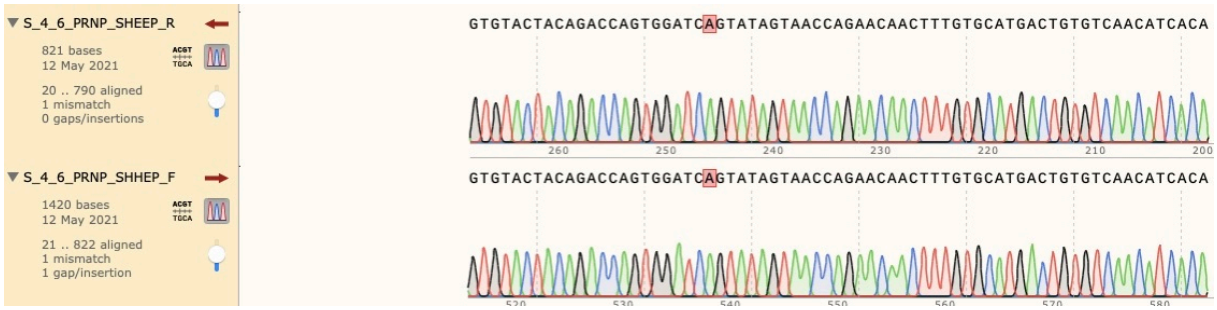
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821 bases
12 May 2021
20 .. 790 aligned
1 mismatch
0 gaps/insertions
S_4_6_PRNP_SHHEP_F
1420 bases
12 May 2021
21 .. 822 aligned
1 mismatch
1 gap/insertion
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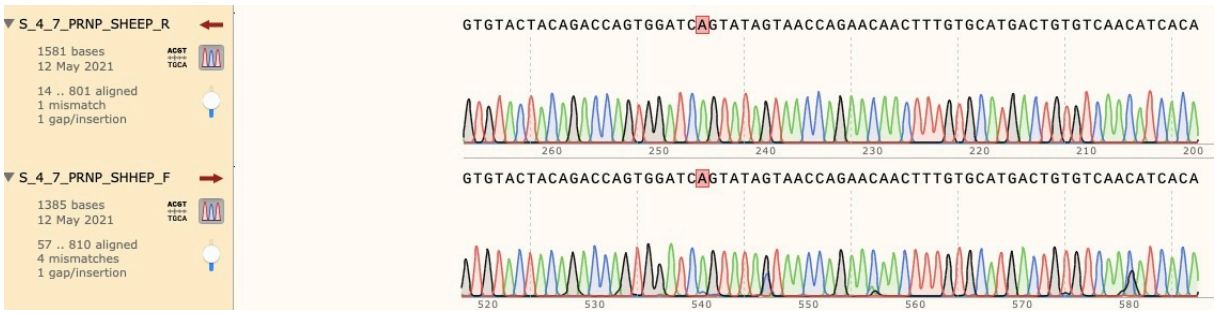
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12 May 2021
14 .. 801 aligned
1 mismatch
1 gap/insertion
S_4_7_PRNP_SHHEP_F
1385 bases
12 May 2021
57 .. 810 aligned
4 mismatches
1 gap/insertion
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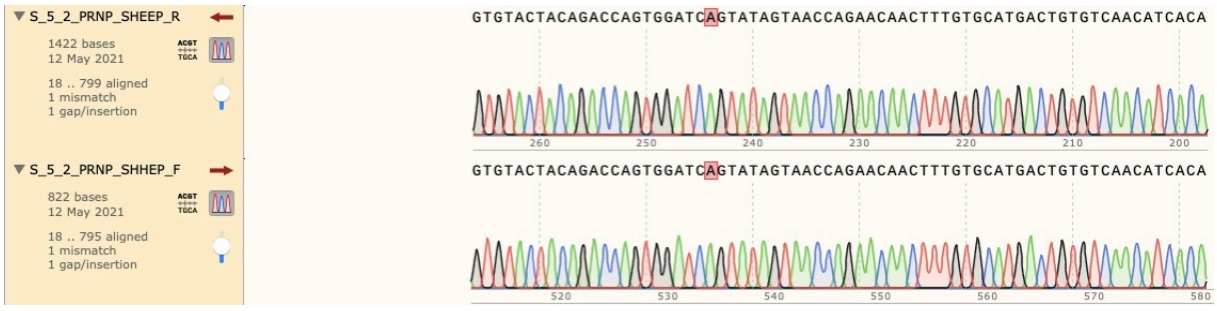
S_5_2_PRNP_SHEEP_R
1422 bases
12 May 2021
18 .. 799 aligned
1 mismatch
1 gap/insertion
S_5_2_PRNP_SHHEP_F
822 bases
12 May 2021
18 .. 795 aligned
1 mismatch
1 gap/insertion
GTGTACTACAGACCAGTGGATCAGTATAGTAACCAGAACAACTTTGTGCATGACTGTGTCAACATCACA
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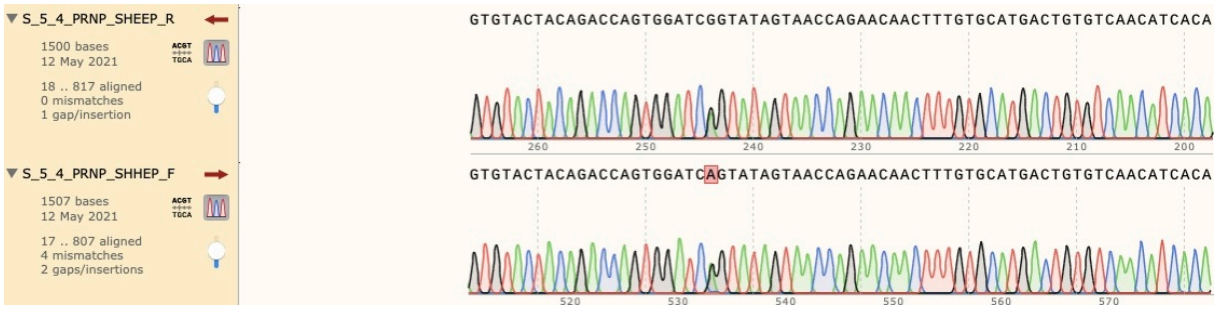
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1500 bases
12 May 2021
18 .. 817 aligned
0 mismatches
1 gap/insertion
S_5_4_PRNP_SHHEP_F
1507 bases
12 May 2021
17 .. 807 aligned
4 mismatches
2 gaps/insertions
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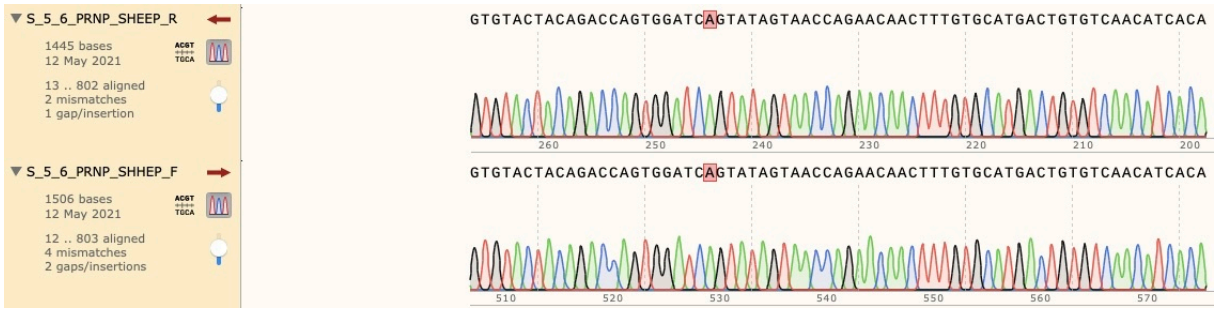
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1445 bases
12 May 2021
13 .. 802 aligned
2 mismatches
1 gap/insertion
S_5_6_PRNP_SHHEP_F
1506 bases
12 May 2021
12 .. 803 aligned
4 mismatches
2 gaps/insertions
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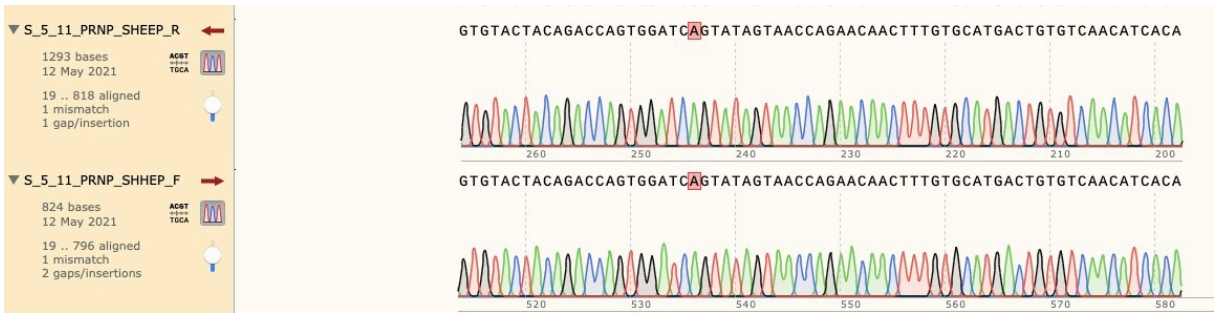
S_5_11_PRNP_SHEEP_R
1293 bases
12 May 2021
19 .. 818 aligned
1 mismatch
1 gap/insertion
S_5_11_PRNP_SHHEP_F
824 bases
12 May 2021
19 .. 796 aligned
1 mismatch
2 gaps/insertions
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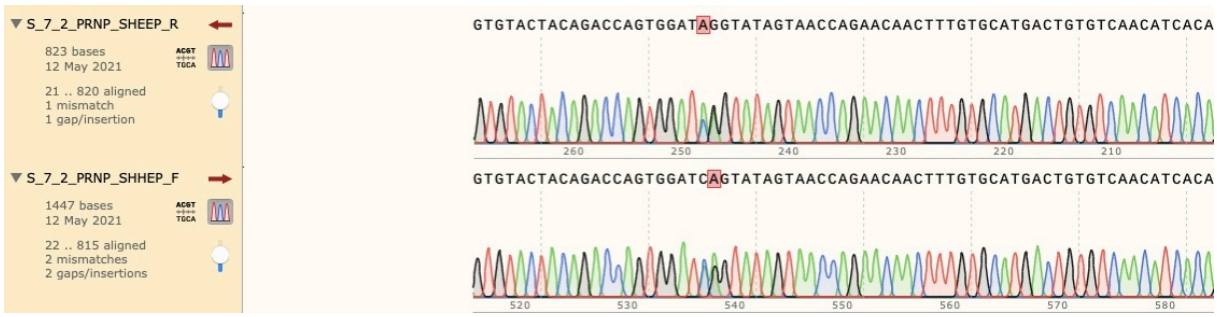
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823 bases
12 May 2021
21 .. 820 aligned
1 mismatch
1 gap/insertion
S_7_2_PRNP_SHHEP_F
1447 bases
12 May 2021
22 .. 815 aligned
2 mismatches
2 gaps/insertions
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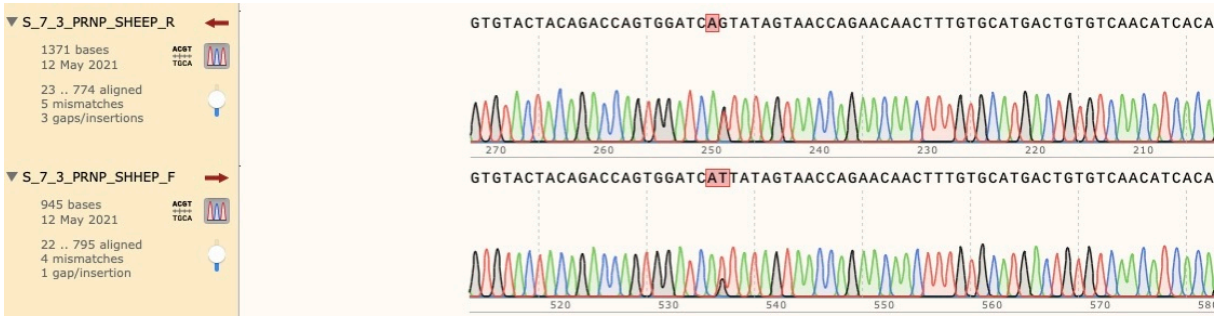
S_7_3_PRNP_SHEEP_R
1371 bases
12 May 2021
23 .. 774 aligned
5 mismatches
3 gaps/insertions
S_7_3_PRNP_SHHEP_F
945 bases
12 May 2021
22 .. 795 aligned
4 mismatches
1 gap/insertion
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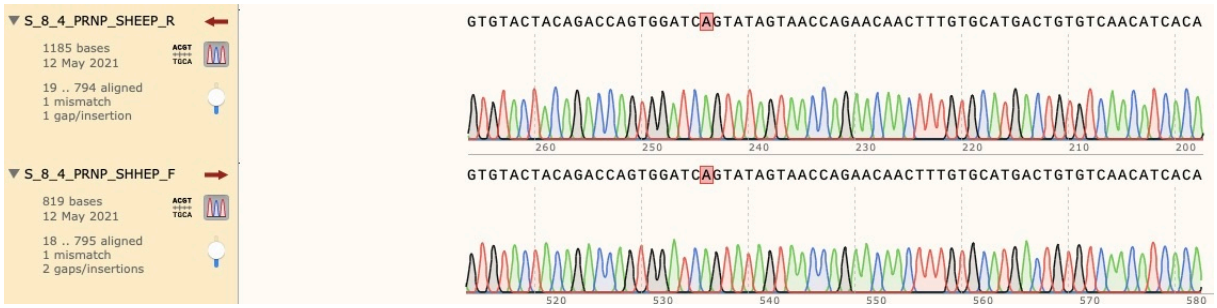
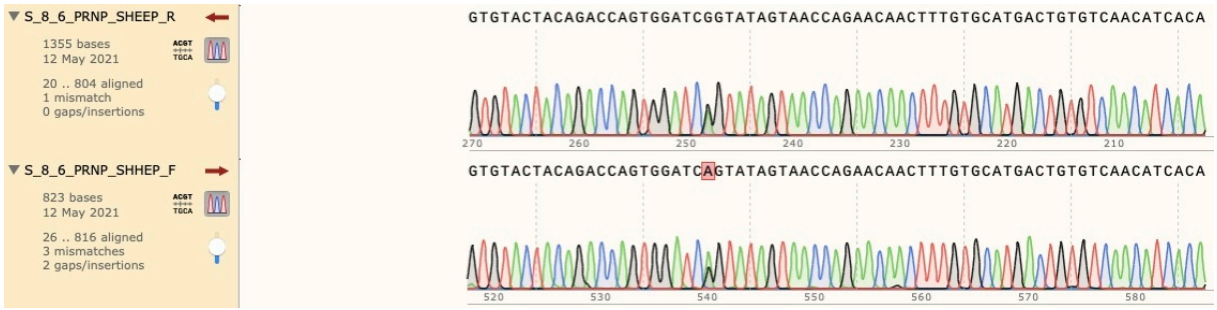
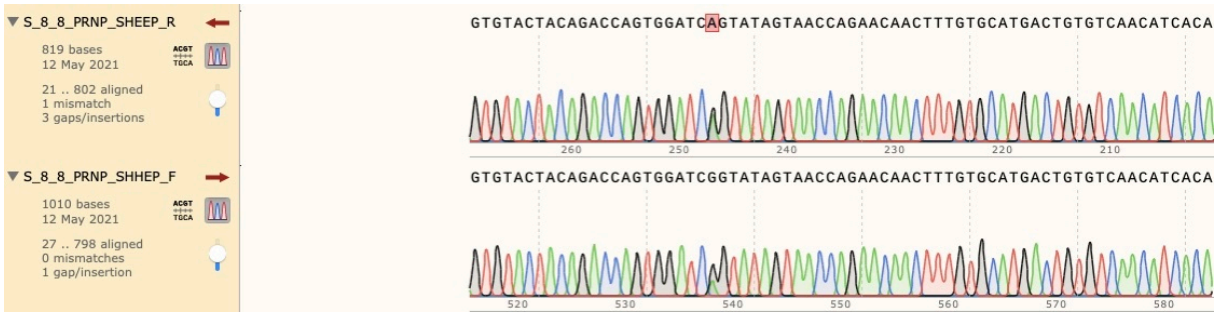
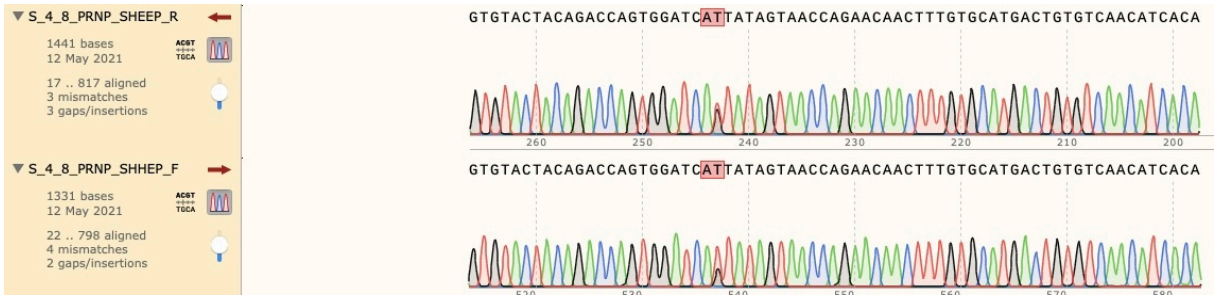
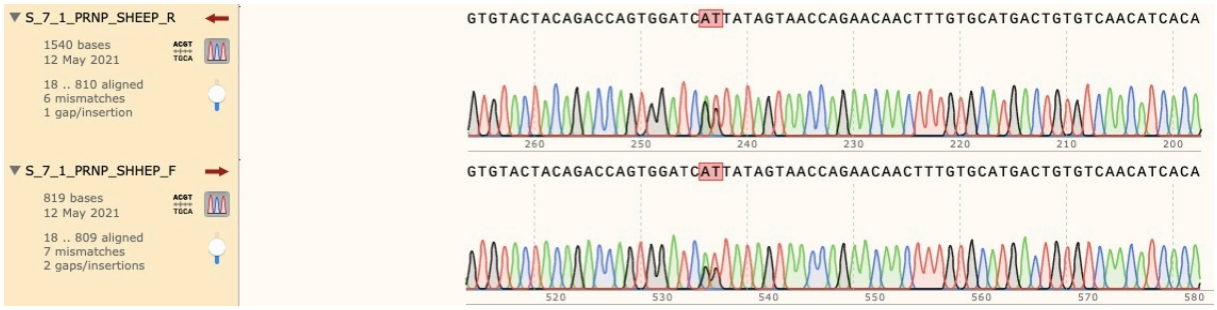


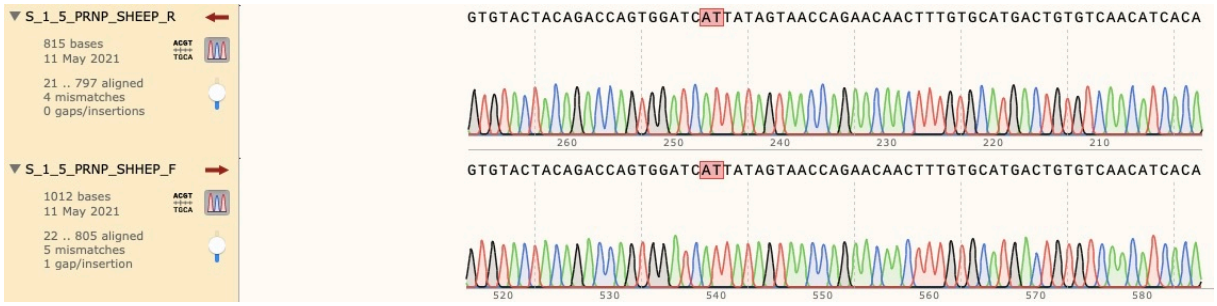


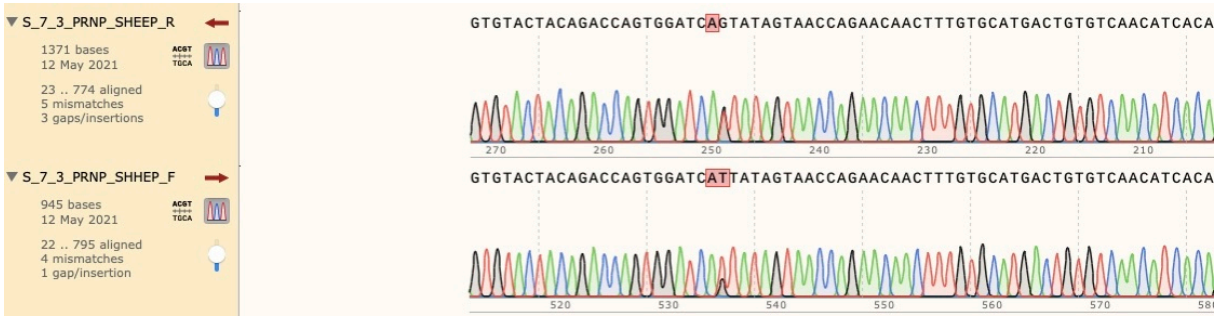


R171H Genotype Sequencing Results

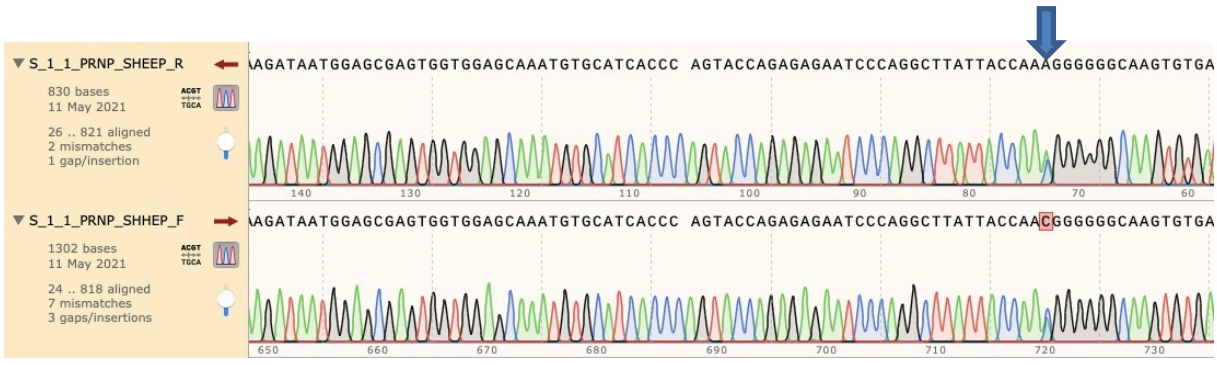


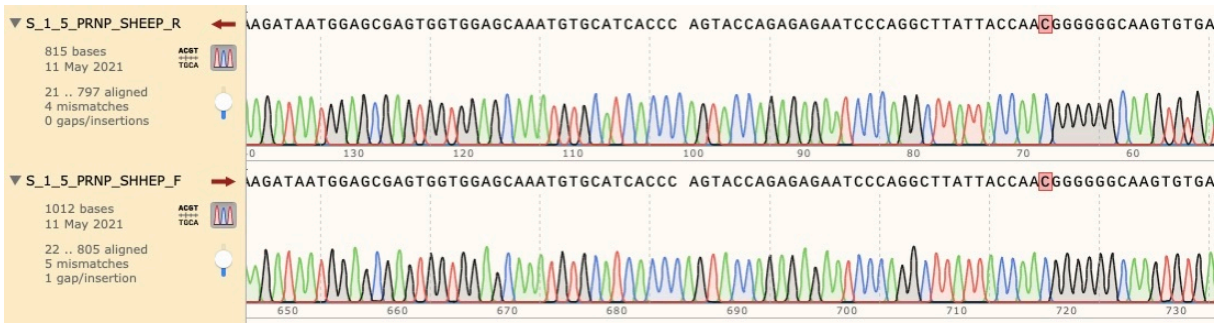






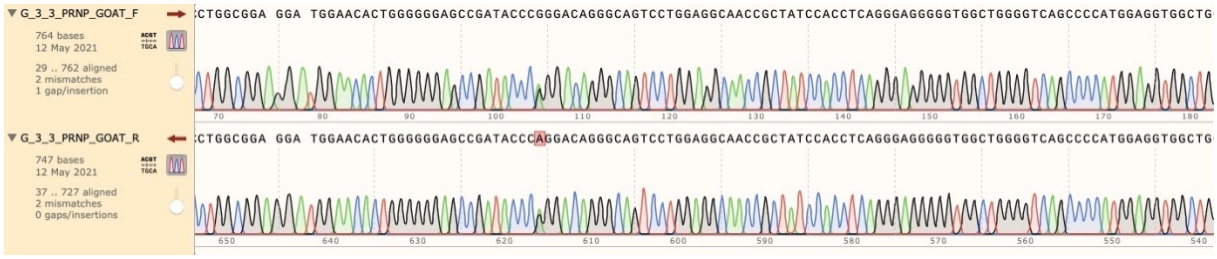
R231T Genotype Sequencing Results

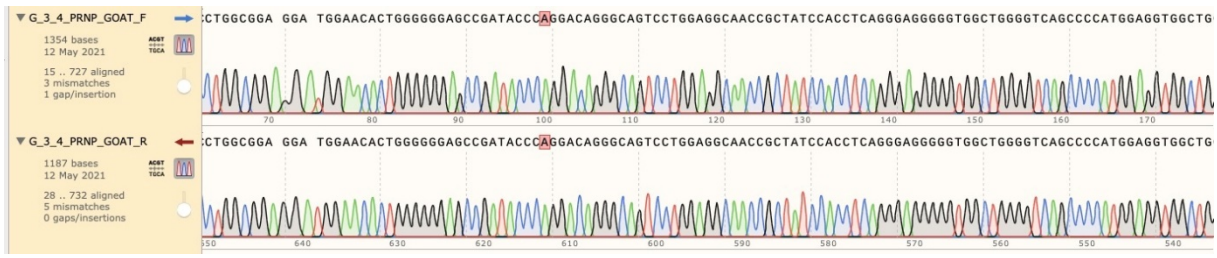


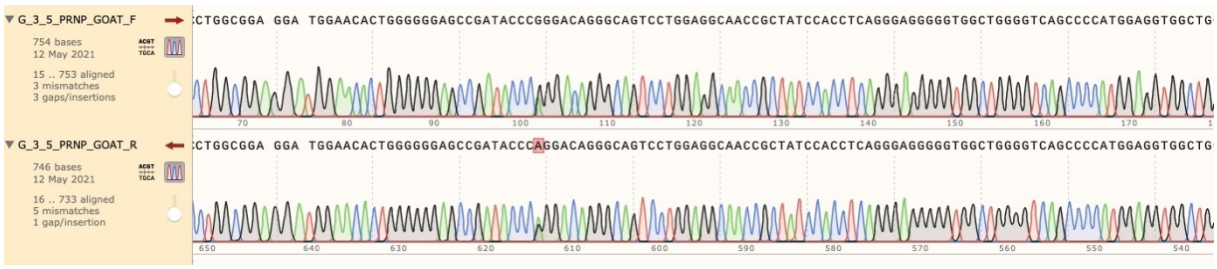


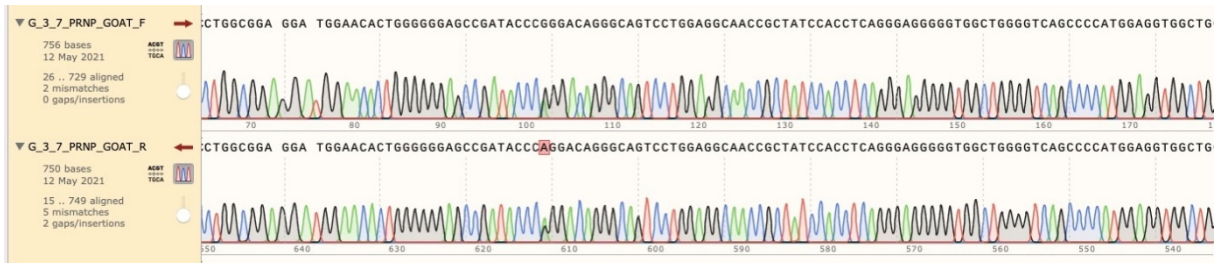
Goat

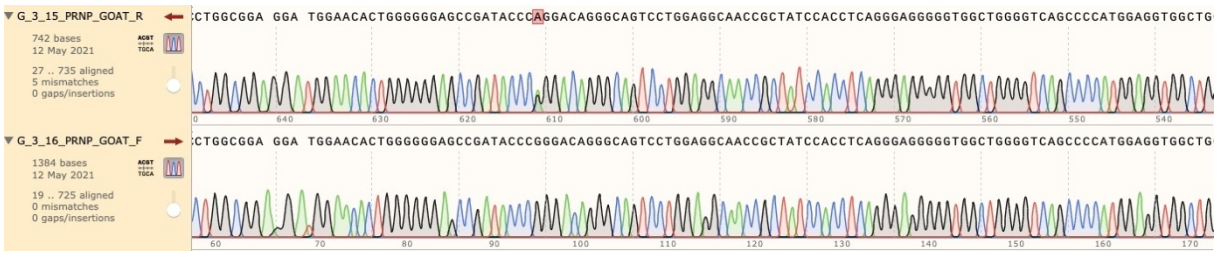
G>A p.P42= (CCG>CCA) Genotype Sequencing Results

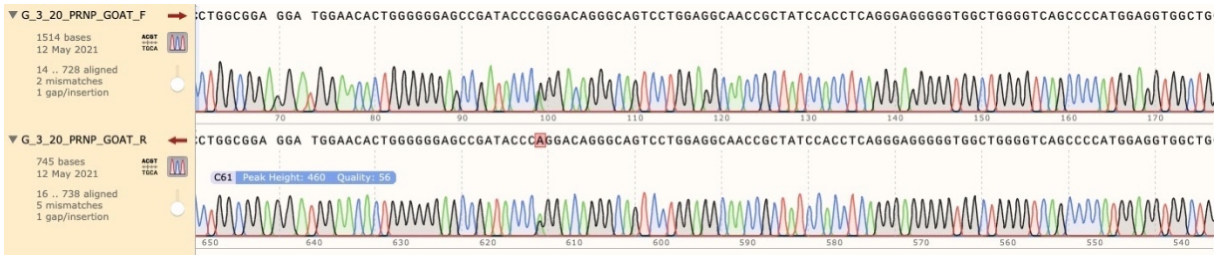

G_3_3_PRNP_GOAT_F
764 bases
12 May 2021
29 .. 762 aligned
2 mismatches
1 gap/insertion
G_3_3_PRNP_GOAT_R
747 bases
12 May 2021
37 .. 727 aligned
2 mismatches
0 gaps/insertions
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG

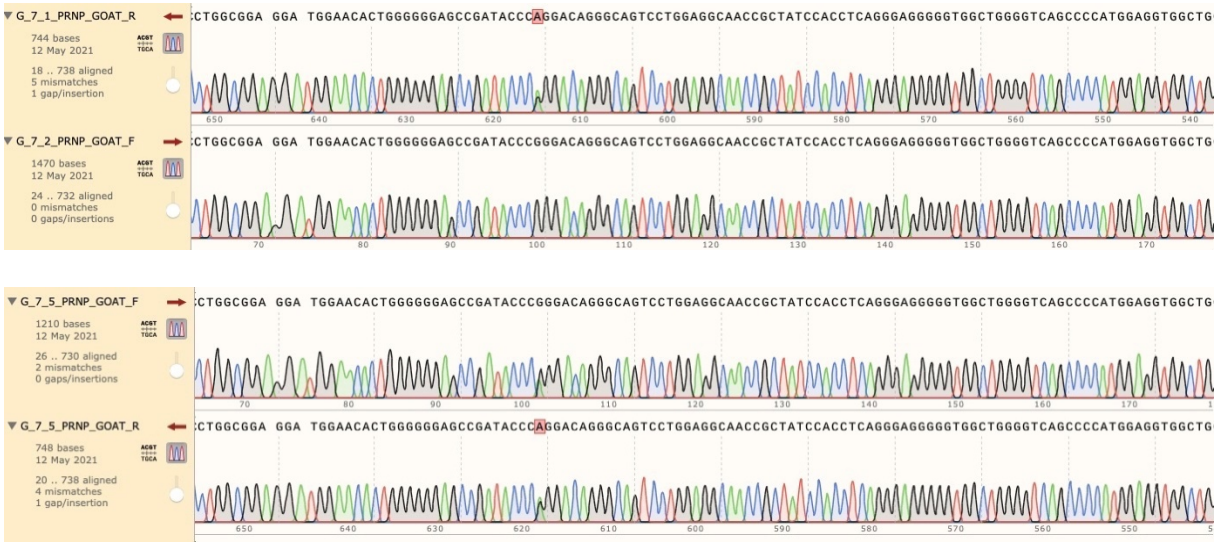

G_3_4_PRNP_GOAT_F
1354 bases
12 May 2021
15 .. 727 aligned
3 mismatches
1 gap/insertion
G_3_4_PRNP_GOAT_R
1187 bases
12 May 2021
28 .. 732 aligned
5 mismatches
0 gaps/insertions
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG


G_3_5_PRNP_GOAT_F
754 bases
12 May 2021
15 .. 753 aligned
3 mismatches
3 gaps/insertions
G_3_5_PRNP_GOAT_R
746 bases
12 May 2021
16 .. 733 aligned
5 mismatches
1 gap/insertion
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG

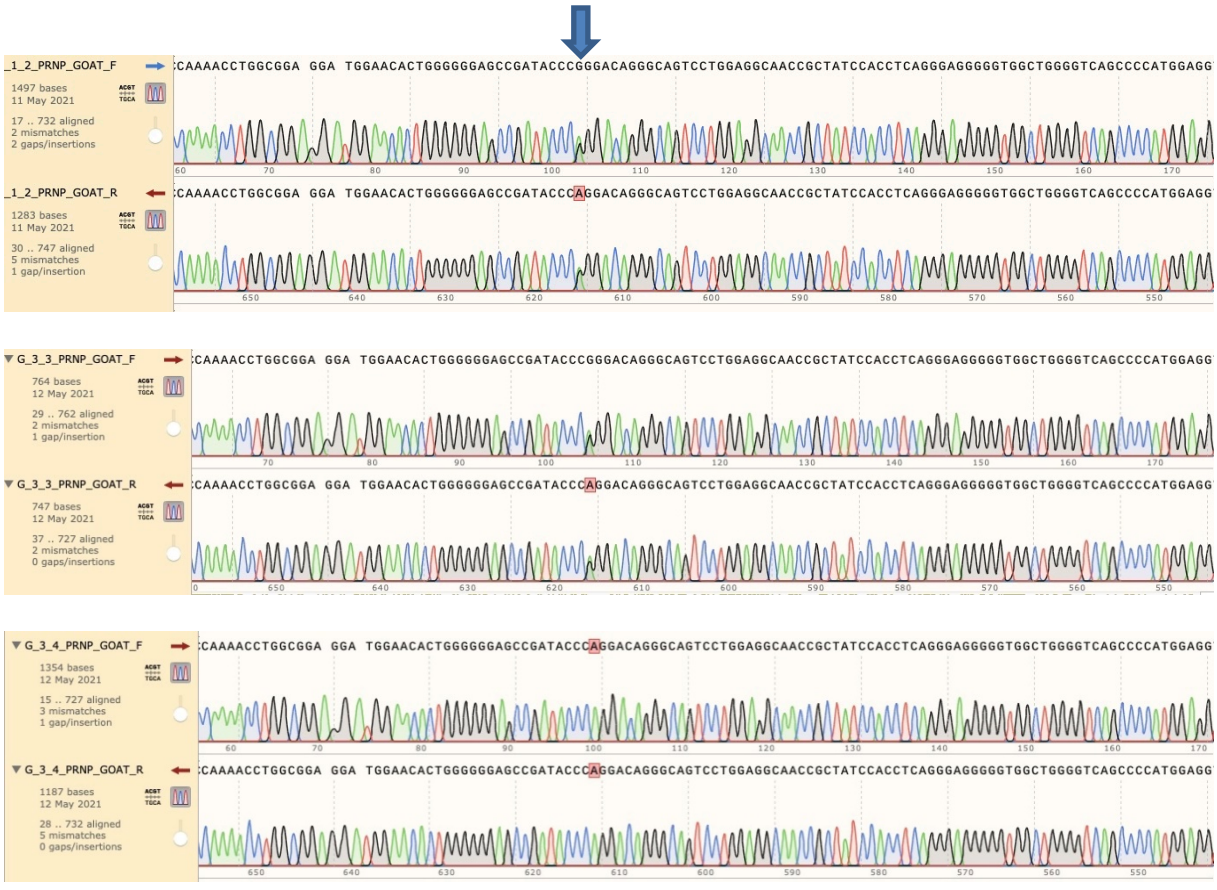

G_3_7_PRNP_GOAT_F
756 bases
12 May 2021
26 .. 729 aligned
2 mismatches
0 gaps/insertions
G_3_7_PRNP_GOAT_R
750 bases
12 May 2021
15 .. 749 aligned
5 mismatches
2 gaps/insertions
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG

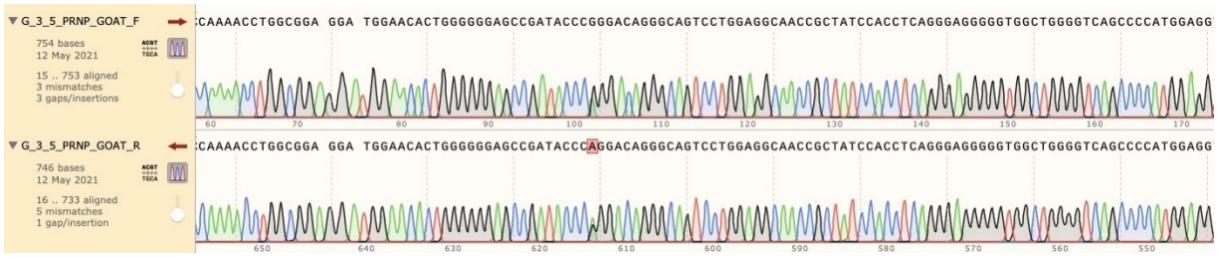

G_3_15_PRNP_GOAT_R
742 bases
12 May 2021
27 .. 735 aligned
5 mismatches
0 gaps/insertions
G_3_16_PRNP_GOAT_F
1384 bases
12 May 2021
19 .. 725 aligned
0 mismatches
0 gaps/insertions
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG

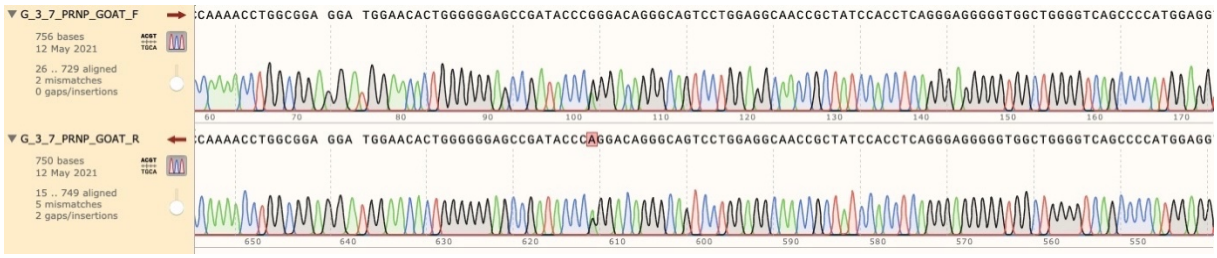

G_3_20_PRNP_GOAT_F
1514 bases
12 May 2021
14 .. 728 aligned
2 mismatches
1 gap/insertion
G_3_20_PRNP_GOAT_R
745 bases
12 May 2021
16 .. 738 aligned
5 mismatches
1 gap/insertion
C61 Peak Height: 460 Quality: 56
CTGGCGGA GGA TGGAACACTGGGGGGAGCCGATACCCAGGACAGGGCAGTCCTGGAGGCAACCGCTATCCACCTCAGGGAGGGGGTGGCTGGGGTCAGCCCCATGGAGGTGGCTG

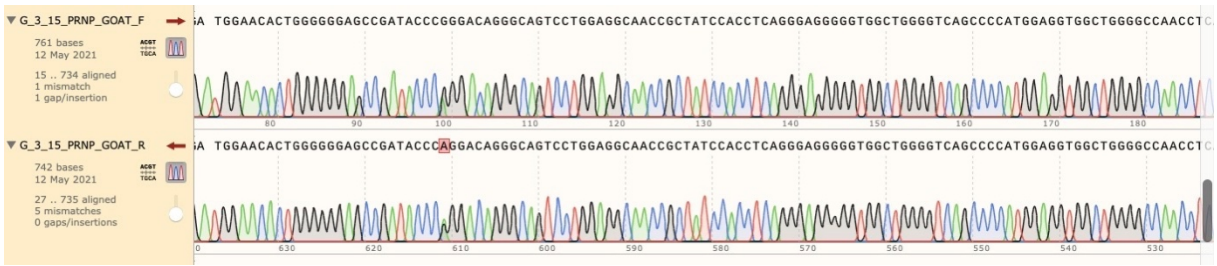


Gly127ser G>A (GGC>AGC) Genotype Sequencing Results

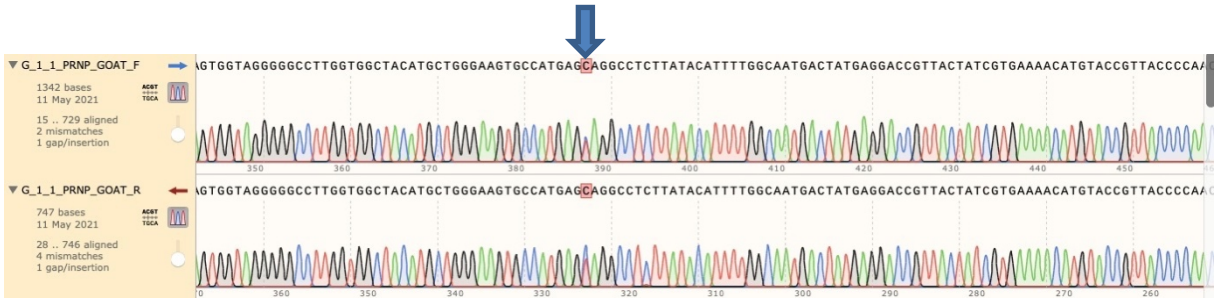


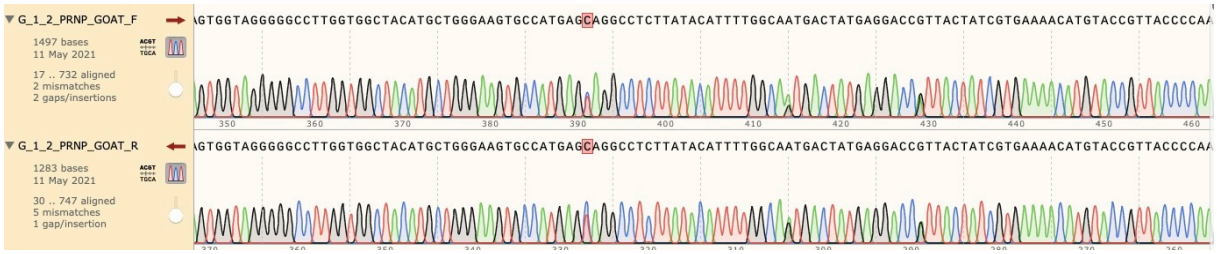


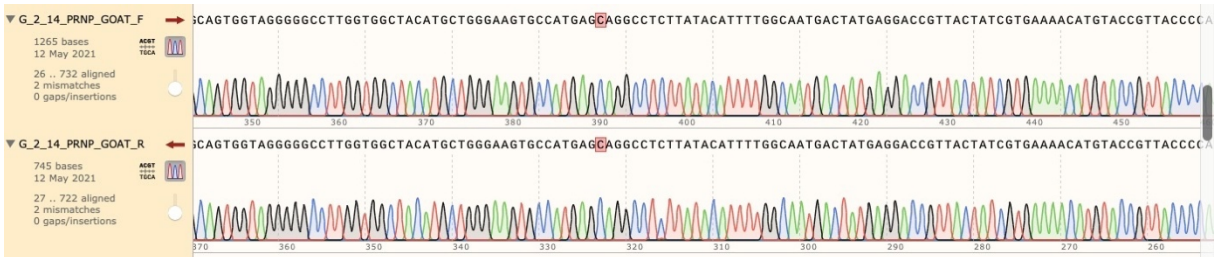


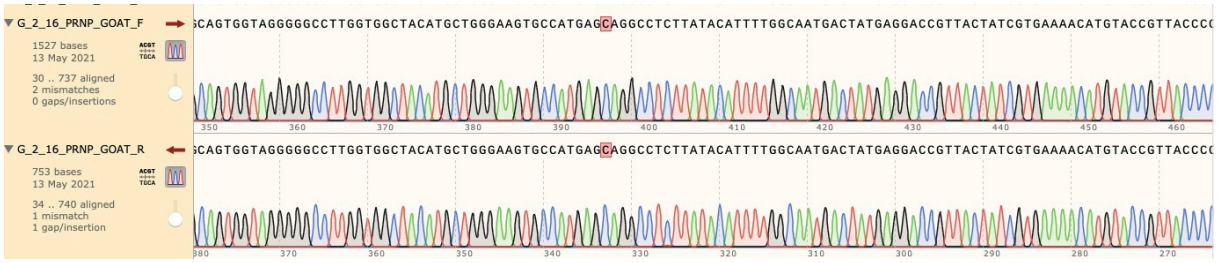


Ser138= (AGT>AGC) Genotype Sequencing Results

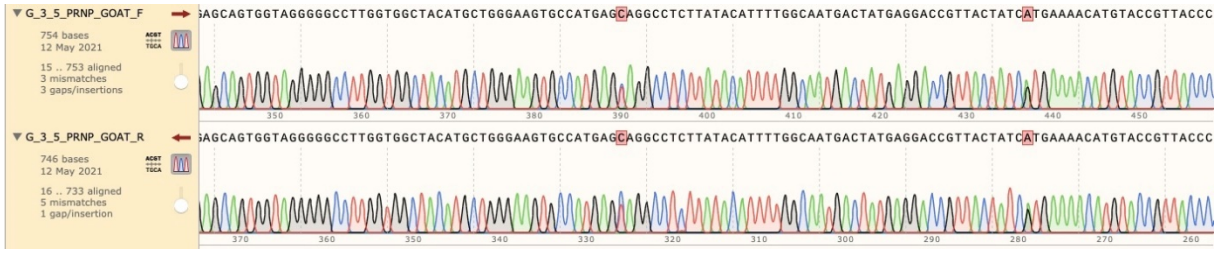


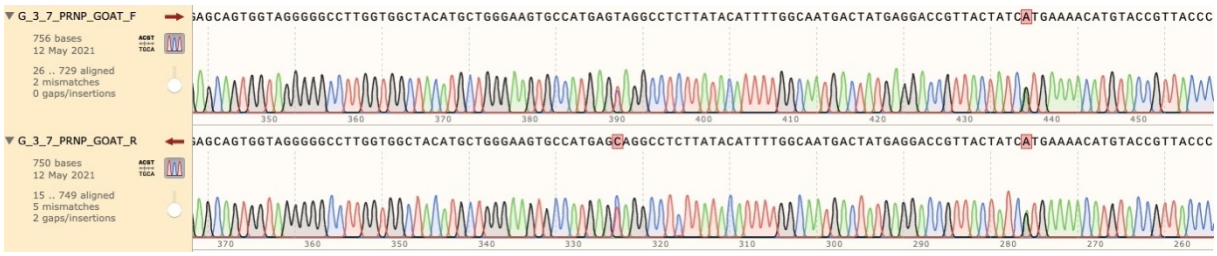


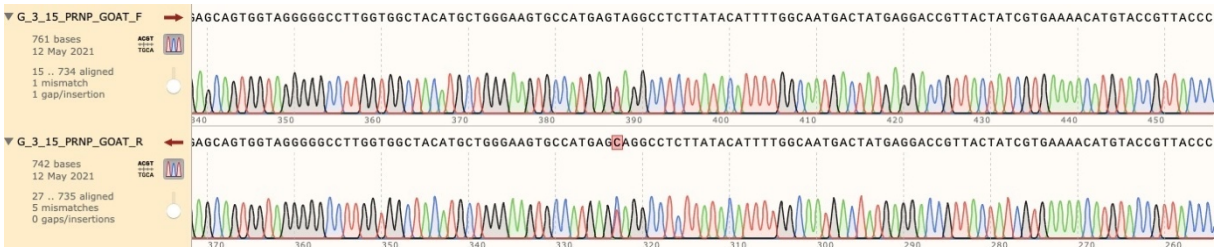


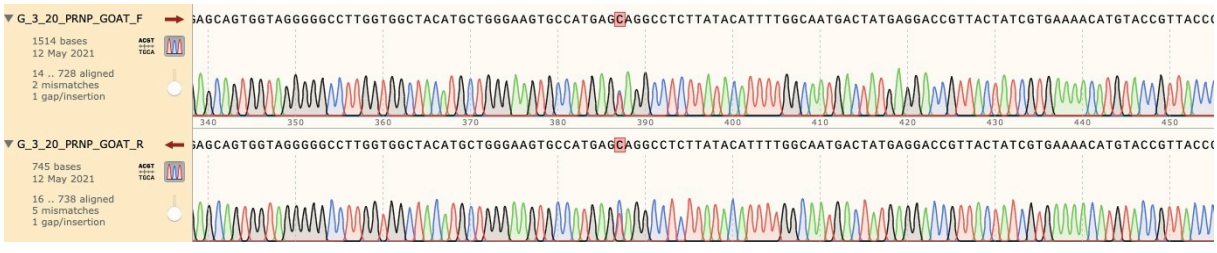

G_2_16_PRNP_GOAT_F
1527 bases
13 May 2021
30 .. 737 aligned
2 mismatches
0 gaps/insertions
G_2_16_PRNP_GOAT_R
753 bases
13 May 2021
34 .. 740 aligned
1 mismatch
1 gap/insertion

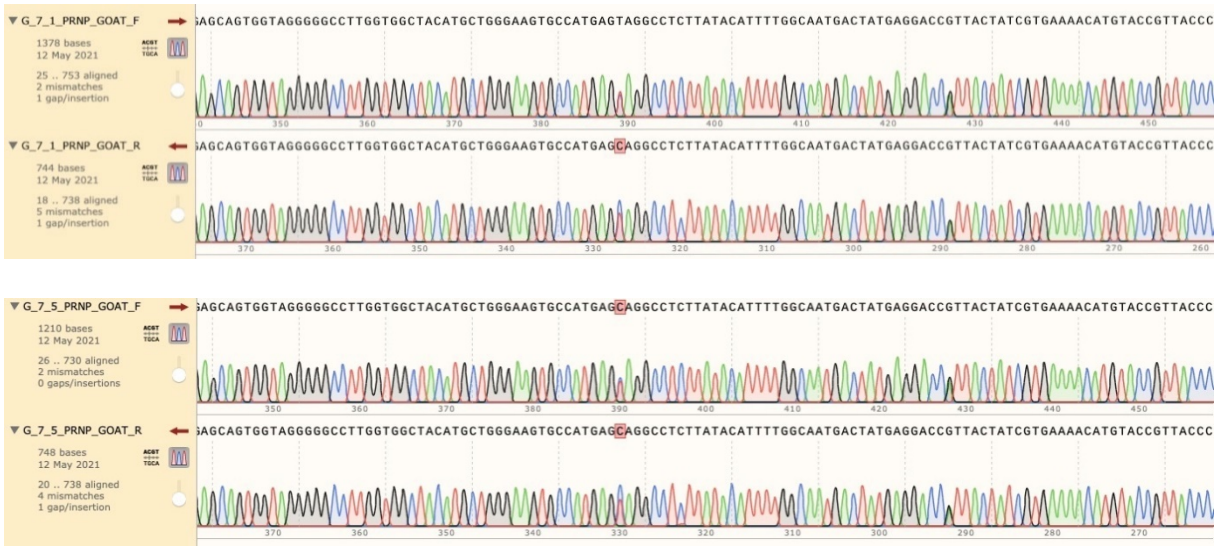

G_3_4_PRNP_GOAT_F
1354 bases
12 May 2021
15 .. 727 aligned
3 mismatches
1 gap/insertion
G_3_4_PRNP_GOAT_R
1187 bases
12 May 2021
28 .. 732 aligned
5 mismatches
0 gaps/insertions


G_3_5_PRNP_GOAT_F
754 bases
12 May 2021
15 .. 753 aligned
3 mismatches
3 gaps/insertions
G_3_5_PRNP_GOAT_R
746 bases
12 May 2021
16 .. 733 aligned
5 mismatches
1 gap/insertion

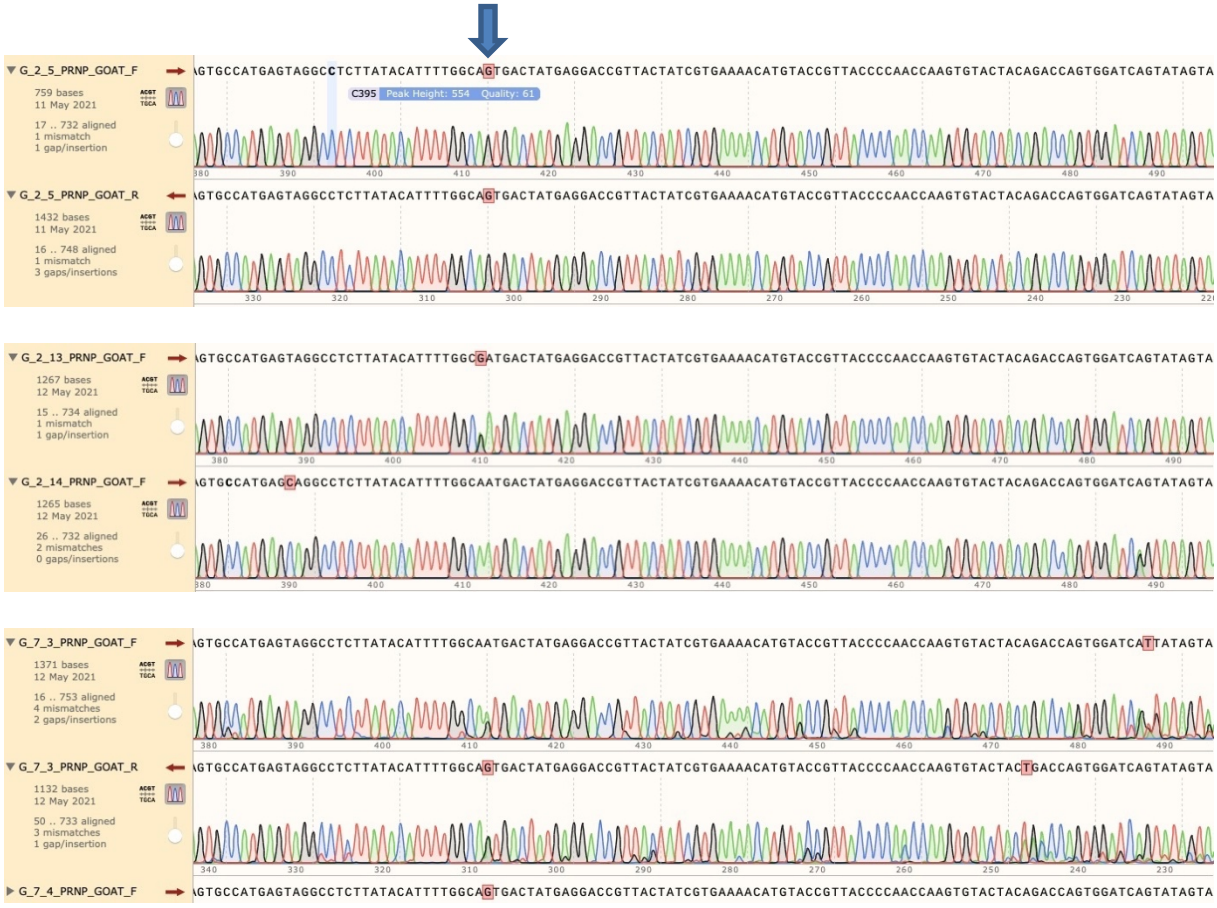

G_3_7_PRNP_GOAT_F
756 bases
12 May 2021
26 .. 729 aligned
2 mismatches
0 gaps/insertions
G_3_7_PRNP_GOAT_R
750 bases
12 May 2021
15 .. 749 aligned
5 mismatches
2 gaps/insertions

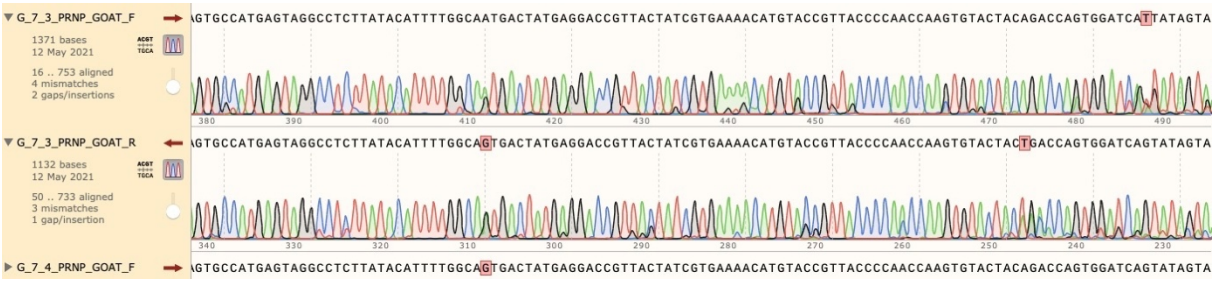

G_3_15_PRNP_GOAT_F
761 bases
12 May 2021
15 .. 734 aligned
1 mismatch
1 gap/insertion
G_3_15_PRNP_GOAT_R
742 bases
12 May 2021
27 .. 735 aligned
5 mismatches
0 gaps/insertions


G_3_20_PRNP_GOAT_F
1514 bases
12 May 2021
14 .. 728 aligned
2 mismatches
1 gap/insertion
G_3_20_PRNP_GOAT_R
745 bases
12 May 2021
16 .. 738 aligned
5 mismatches
1 gap/insertion

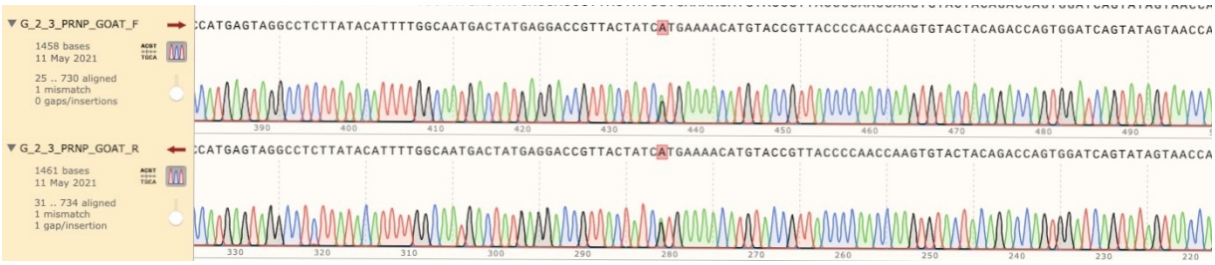


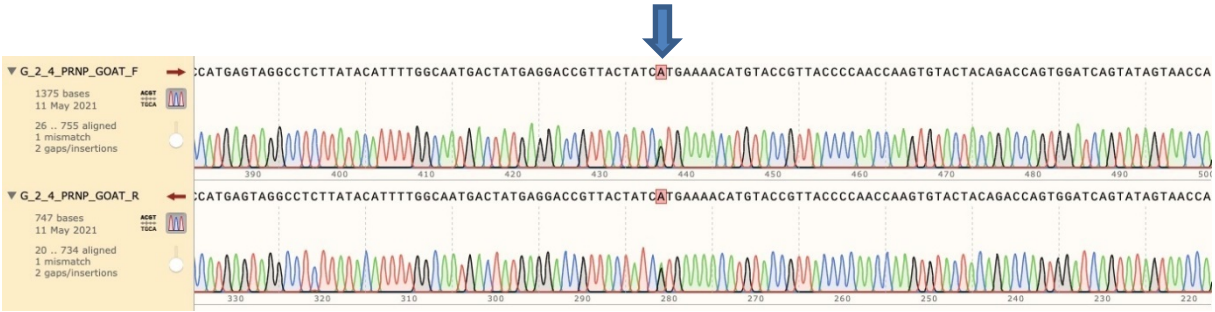
Asn146Ser (AAT>GAT) Genotype Sequencing Results

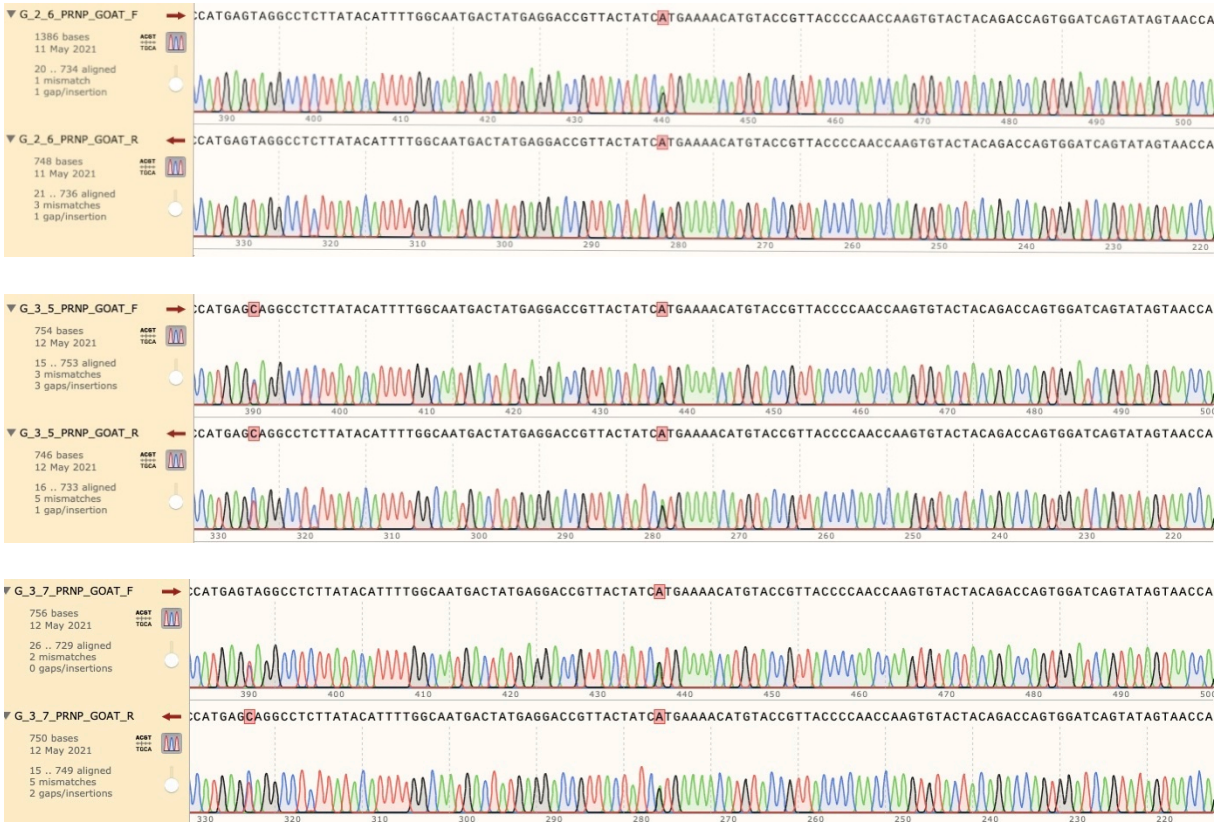




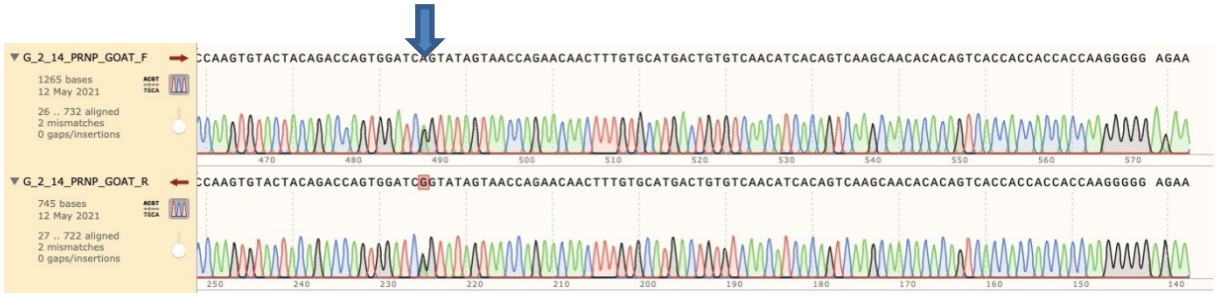
Arg154His (CGT>CAT) Genotype Sequencing Results



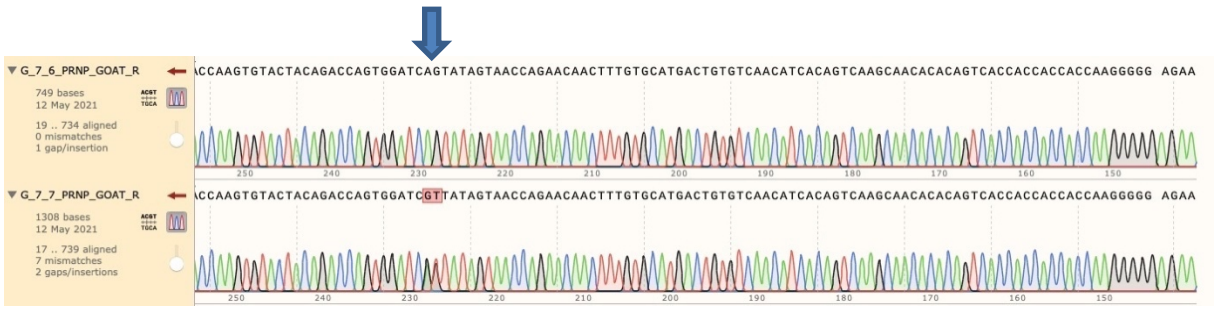




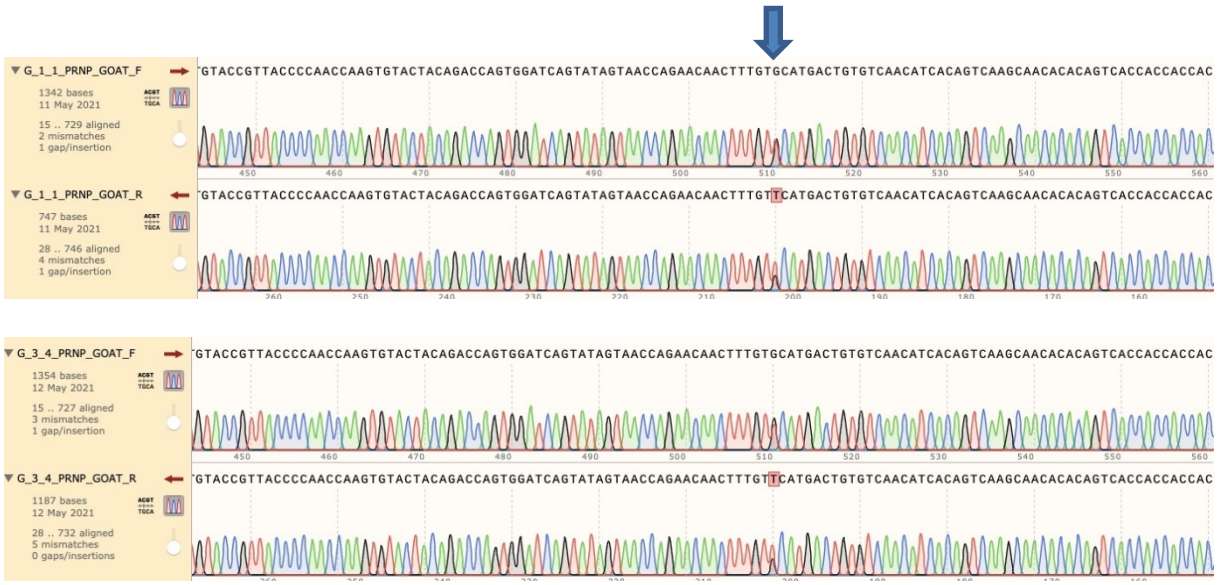
Gln172Arg (CAG>CGG) Genotype Sequencing Results



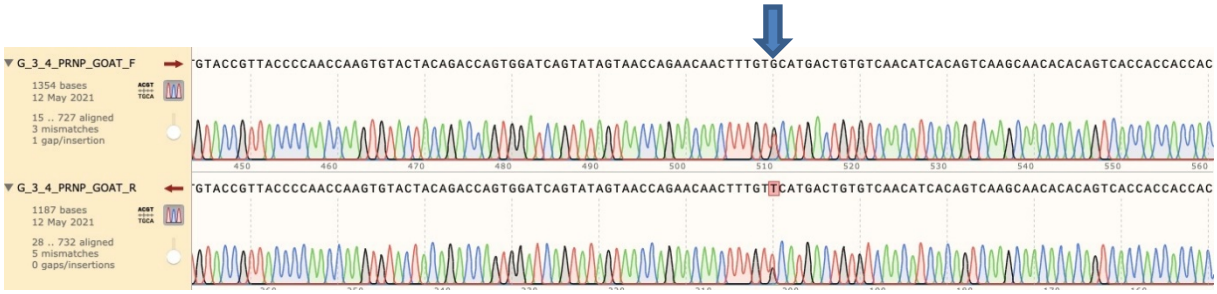
Gln172His (CAG>CAT) Genotype Sequencing Results



Val179Val (CAG>CAT) Genotype Sequencing Results

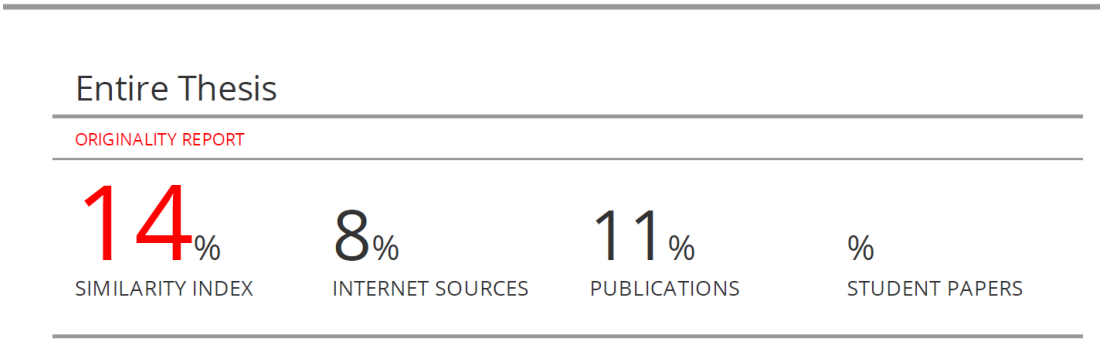


Val187Val (CAG>CAT) Genotype Sequencing Results



Appendix B

Signed Similarity Report



Assoc. Prof. Mahmut Çerkez Ergören

Appendix C
Curriculum Vitae

PERSONAL INFORMATIONS

Surname, Name:	Betmezoğlu, Meryem
Date of Birth:	2 April 1990
Place of Birth:	Nicosia, Cyprus

EDUCATION

Degree	Department/Program	University	Year of Graduation
B.Sc.	Veterinary	Near East University Veterinary Fakulty	2017

WORK EXPERIENCE

Title	Place	Year
Research Assistant	NEU, Faculty of Veterinary, Department of Genetic	2017-present

PUBLICATIONS IN INTERNATIONAL REFERED JOURNALS

- Betz Heinemann, K. A., Betmezoğlu, M., Ergoren, M. C., & Fuller, W. J. (2020). A murder of crows: culling corvids in Northern Cyprus. *Human Ecology*, *48*(2), 245-249.
- BETMEZOĞLU, M., & ARSOY, D. The Present of Scrapie and the Result of Breeding Program in Europe and Cyprus. *Black Sea Journal of Engineering and Science*, *2*(1), 33-38.