

HISTORY OF PRION PROTEIN GENE (*PRNP*) POLYMORPHISM IN SHEEP AND SCIENTIFIC FINDINGS

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Abstract. Scrapie is basically a kind of disease that originally was specific to European countries, but from England it spread all over the world to Canada, South Africa, Australia, New Zealand and many other countries. Scrapie is a prion disease which is fatal and results in or can be characterized by the degeneration of the nervous system. It belongs to transmissible spongiform encephalopathies (TSEs) infecting small ruminants including sheep and goat. Sheep susceptibility or resistance to classical scrapie is highly supervised by the polymorphisms at codons 136, 154 and 171 of the *PRNP*. In this review, we found that countries like Romania, Finland, Italy, Slovakia, Germany, Greece, Spain, Poland, Turkey, Iran, Brazil, England, Portugal, Hungary, Austria, and Czech Republic, are susceptible to scrapie, while in Pakistan, China, Algeria, West Africa, America, Burkina Faso, and Niger are those countries where sheep are not susceptible to this disease. From these studies, we can clearly conclude that China and Pakistan are the countries where sheep show more resistance to scrapie. We focused to summarize the *PRNP* polymorphism at 136, 154, and 171 in sheep and some important findings in major parts of the world.

Keywords: *scrapie, transmissible spongiform encephalopathies (TSEs), European countries, history, prion*

Introduction

Approximately, 70.7% of the total sheep population is present in Asia and 35.89% of the total population of sheep is present in China which has become the leading country for sheep products. But in China, no case of scrapie in sheep has been found so far, whereas in the countries like England, Greece, Turkey and other small European countries scrapie is on red alert. Where sheep (*Ovis aries*) and goat (*Capra aegagrus hircus*) have many resemblances, their scientific taxonomy eventually split, have distinct species and genus, whereas sheep and goat have 54 and 60 chromosomes simultaneously. The polymorphism of the *PRNP* gene plays a fundamental role in prion disease. Scrapie is resulting in or characterized by the degeneration of the nervous system affecting sheep and belongs to a group of a prion disease that naturally occurs in sheep. *PRNP* is responsible for negative influence on the financial loss, such as the cashmere yield, wool thickness (Lan et al., 2012) and milk yield (Vitezica et al., 2013) as well as the waistline, body length (Yang et al., 2016), rump length (Yang et al., 2018), and weight. Almost the same mutation of *PRNP* present in sheep, has also been determined in goat (White et al., 2008; Zhou et al., 2013). The only responsible factor for scrapie that has been determined until now is a prion. Scrapie is not like a viral or bacterial disease which have causal agent, rather it is a genetical disease making it difficult to cure. The possibility for the elimination of this disease is wholly associated with polymorphisms of the *PRNP*. Sheep and goat are not the only victims of prion disease as it has been reported in almost all vital living species of animals with different

names (*Table 1*). The first human victimization to scrapie were the farmers or the sheep owners who were directly affected by the disease. During the 20th century, many ideas on the nature of the causative agent of TSEs were published (*Table 2*) while with the passage of time, the majority of these revealed to be unwarranted. Prion disease affects both animals and humans (Yaman and Ün, 2017) but until 1990s scientists failed to provide any evidence of transmission of disease to humans (Van Duijn et al., 1998).

Table 1. Affected species with prion diseases (Mabbott, 2017)

Disease	Species
Scrapie	Sheep, goats, mouflon
Iatrogenic Creutzfeldt-Jakob disease (CJD)	Human
Sporadic Creutzfeldt-Jakob disease	Human
Variant Creutzfeldt-Jakob disease	Human
Familial Creutzfeldt-Jakob disease	Human
Gerstmann-Straussler-Scheinker syndrome	Human
Kuru	Human
Fatal familial insomnia	Human
Bovine spongiform encephalopathy	Cattle
Chronic wasting disease	Elk, deer, moose
Transmissible mink encephalopathy	Mink
Feline spongiform encephalopathy	Domestic and zoological cats
Exotic ungulate encephalopathy	Nyala, kudu

Table 2. Names of causative agents given by different scientists from 1912 to 1991

Year	TSE agents	Reference
1914	Sarcosporidia	M'Gowan (1914)
1938	A filterable virus	Cuillé and Chelle (1938)
1954	A slow virus	Sigurdsson (1954)
1966	A replicating polysaccharide	Alper et al. (1967)
1967	A protein	Pattison and Jones (1967)
1967	A replicating membrane	Gibbons and Hunter (1967)
1968	A DNA-polysaccharide complex	Adams and Caspary (1968)
1972	A viroid	Diener (1972)
1978	A lipid	Alper et al. (1978)
1979	A Spiroplasma sp.	Bastian (1979)
1979	A virino	Dickinson (1979)
1982	A prion	Prusiner (1982)
1984	A virus	Manuelidis (1996)
1989	Mitochondria (l nucleic acid(s))	Aiken et al. (1990)
1991	A holopron, consisting of abnormal prp (PrP in the scrapie specific conformation, the apopron) and a (dispensable) nucleic acid (the copron)	Weissmann (1991)

Scrapie is the disease with the maximum and oldest publications as explicit journals allude to a paper going back to the year 1732 as the initial report of scrapie (Detwiler and Baylis, 2003). In 1772 scrapie was accounted for to be known for around 40 years, a point in time going back to the year 1732. This distemper disease is normally said to be remains of almost forty years in England (Comber, 1772). All scholars were devoted to the quest for the origin of the disease. A large number of proposed causes were diagnosed by all methods like the number of prescribed pathogens set forward all through the twentieth century. Since the 1930s, scrapie examination was reinforced when impressive money related misfortunes to the sheep business were brought about by expanding measurements of cases. These harms likewise advance studies on the precise idea of the infective reason, besides parasites (M’Gowan, 1914) and bacteria (Bastian, 1979) as causative agents, virus infection was the most frequently proposed principle, already formulated in 1938 (Cuillé and Chelle, 1938). In 1954, the word of a “slow virus infection” was presented the first time (Sigurdsson, 1954). Though, in 1966, a substitute to the viral origin was hypothesized as the cause, i.e., polysaccharides (Alper et al., 1966; Alper et al., 1967) or lipids (Alper et al., 1978). In 1967, for the first time, a protein was predictable as an infective cause (Pattison and Jones, 1967), and the first “protein-only- hypothesis” was articulated (Griffith, 1967), which was followed in the 1970s by the “virino” theory (Dickinson, 1979). At long last, in light of the opposition of the pathogen, in 1982, “proteinaceous irresistible molecule” (abbreviation: prion) was presented (Prusiner, 1982) and the transformation of a healthy cellular protein (PRPC) into a pathological isoform (PRPSc) as a critical event of TSE pathogenesis was proposed not long after (Oesch et al., 1985).

The investigation of scrapie was complex by the circumstance that in previous times, many new diseases like Drehkrankheit, Kreuzdrehe, and Gnubberkrankheit were confused with scrapie. Numerous scholars believed at least one of them to be indistinct with or separate from scrapie. Some particular authors attempted to recognize “Drehkrankheit,” “Gnubberkrankheit,” “Kreuzdrehe,” and “Traberkrankheit.” Whereas a lot of them segregate among “Drehkrankheit” and “Kreuzdrehe” from one viewpoint and “Traberkrankheit” on the other (Frank, 1820; Ribbe, 1821; Hering, 1849; Erdt, 1861; May, 1868), there were other writers who considered “Kreuzdrehe” and “Traberkrankheit” to be the identical but to be dissimilar from “Gnubberkrankheit” (Wagenfeld, 1829). This mistake of terms, just as the indistinct and confounding portrayal of the indications of scrapie and of different diseases, indicating scrapie recognized in the year 1750. Different terms that were utilized to make reference to scrapie are mentioned (*Table 3*).

Table 3. Historical names of scrapie given by locals in different regions of the world

	Name of scrapie	Country	Reference
1	Basquilla Disease	Spain	von Richthofen (1828)
2	Cuddie Trot	Scotland	Healy et al. (2003)
3	Drab(en)	Germany	von Richthofen (1827)
4	Dreb/Deeb	Germany	Frank (1820)
5	Drehkrankheit	Germany	Schneider et al. (2008)
6	Gaubber/G(n)aup(p)er	Germany	Schneider et al. (2008)
7	Gnubberkrankheit	Germany	Cassirer (1898)
8	Knopper	Germany	Frank (1820)

9	Khujali	India	Katiyar (1962)
10	Kreu(t)zdrehe(n)	Germany	von Richthofen (1827)
11	Kreutzschlagen	Germany	Albert and Brunn (1818)
12	La maladie convulsive	France	Liberski and Jaskólski (2002)
13	La maladie foll(i)e	France	Beck et al. (1964)
14	La maladie trotteurs	France	Besnoit (1899)
15	La prurigo lombaire	France	Liberski and Jaskólski (2002)
16	La Tremblante	France	Beck et al. (1964)
17	Mukoo	India	Katiyar (1962)
18	Petermännchen	Germany	Erdt (1861)
19	Prurigo lombaire	France	Besnoit (1899)
20	Prurigo lumbar	Spain	Yam (2003)
21	Reiberkrankheit	Germany	Beck et al. (1964)
22	Reiber-Uebel	Germany	von Richthofen (1827)
23	Rickets	England	Beck et al. (1964)
24	Rida	Iceland	Pálsson (1979)
25	Rub/Rubbers	England	Beck et al. (1964)
26	Rubbing disease	England	Parry (1983)
27	Ruppe	Germany	Frank (1820)
28	Scabies dorsalis	Germany	Hörnlimann et al. (2001)
29	Schrucken/Schru(c)kigsein	Germany	Frank (1820)
30	Scratchie	Scotland	Liberski and Jaskólski (2002)
31	Shakings	England	Beck et al. (1964)
32	Shrewcroft	England	Liberski and Jaskólski (2002)
33	Shruginess	England	Parry (1983)
34	Spruckigkeit	Germany	Schneider et al. (2008)
35	Tempermänner	Germany	Erdt (1861)
36	Trab(en)/Traberkrankheit	Germany	Beck et al. (1964)
37	Trotting disease	England	Schneider et al. (2008)
38	Trze sawka	Poland	Liberski and Jaskólski (2002)
39	Wetzkrankheit	Germany	May (1868)
40	Yeukie pine	Scotland	Healy et al. (2003)
41	Zitterkrankheit	Germany	May (1868)

The description of literature is that scrapie was firstly born in Europe in the 18th century but until now the presence of scrapie in Europe is a dangerous sign for all over the world due to the threat of its spreading (*Fig. 1*) from England to all over the world (Detwiler and Baylis, 2003). The evidence of the scrapie in the European Union and the nearby regions from the day first till 2016 (EFSA, 2017) were as follows.

In 1987, 442 animals infected with scrapie were reported in England. In 1992, the number of animals infected with scrapie were reported as 37301. This number was reported as 1123 in 2002 and 610 animals in 2013, after that there were no reports of animals infected with scrapie. In Ireland, 15 animals were reported in 1990, in 2002, a total of 334 animals were reported, and in 2017, the disease showed peak time. Scrapie disease in France was first published in 1993, in 2002, 240 patients were reported. The first case was reported in Germany in 1991, the number of animals

reported in 2002 were 7, while in 2001, 125 animals were published (EFSA, 2017). The first case of scrapie in Spain was reported in 1987, and in 2001, it was observed that the number of infected animals had been increased (Acín et al., 2004). Spain has started to work on the genotypic characterization of various races in this sense, to develop different strategies for each race and to prepare the laws governing these programs (Ugarte and Gabina, 2004). The first case of scrapie in Greece was settled in the north of the country in 1986, and the second case was diagnosed in 1997, the latter case was diagnosed after 11 years. The second case was seen near the region where the first case was observed, which was the evidence that the implemented eradication program is inadequate (Leontides et al., 2000). In 2001, there were 18 cases reported in Greece. According to the eradication program in Greece, the herds with the disease were massacred (Billinis et al., 2004). As a result of the breeding policies observed in Europe, the number of scrapie cases has decreased since 2009 (EFSA, 2014). In sheep, 933 scrapie cases were reported in the EU in 2017, which is an increase of 36.2% compared with 2016 (EFSA, 2018). In *Figure 2*, Cosseddu showed us the presence of scrapie in all over the world in 2007.

Resistance and susceptibility

Sheep can be resistant and susceptible to the disease and the majority of susceptible sheep are in European countries. Every one of these discernments lead to the European Union (EU) keeping on developing breeding projects focused at developing the frequency of safe alleles in breeds from all member-states. These European programs are established on five groups of genotypes, from extremely resistant to extremely sensitive, whose alleles ARR and VRQ are considered highly resistant and susceptible to scrapie, respectively (Hunter, 1997).

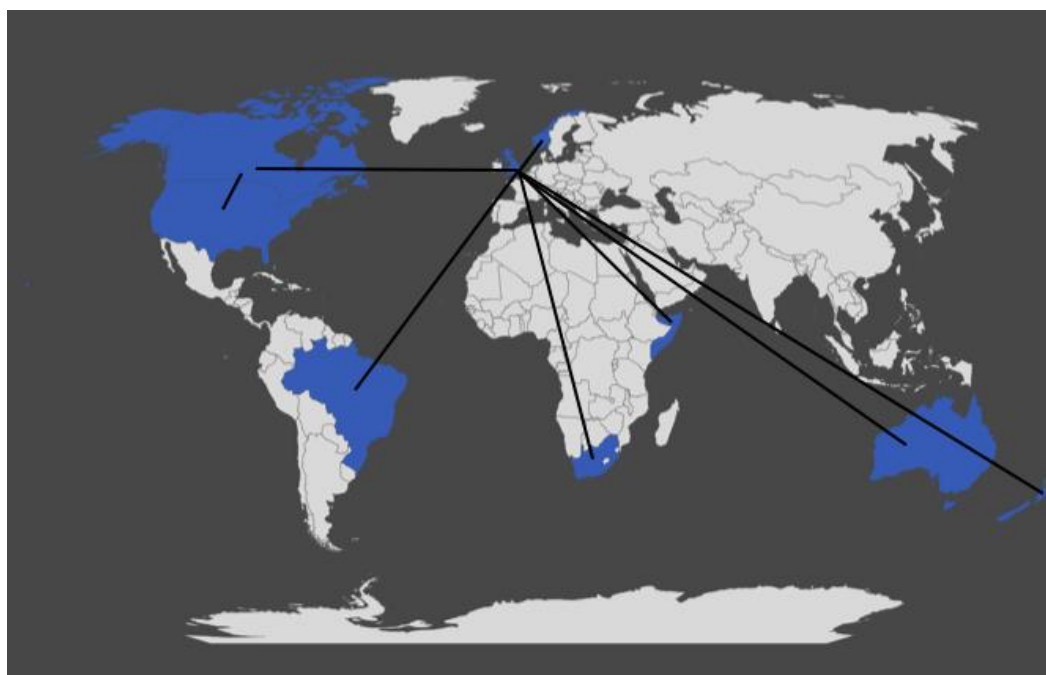


Figure 1. Spread of scrapie from England to the major parts of the world in 2003 (Detwiler and Baylis, 2003)

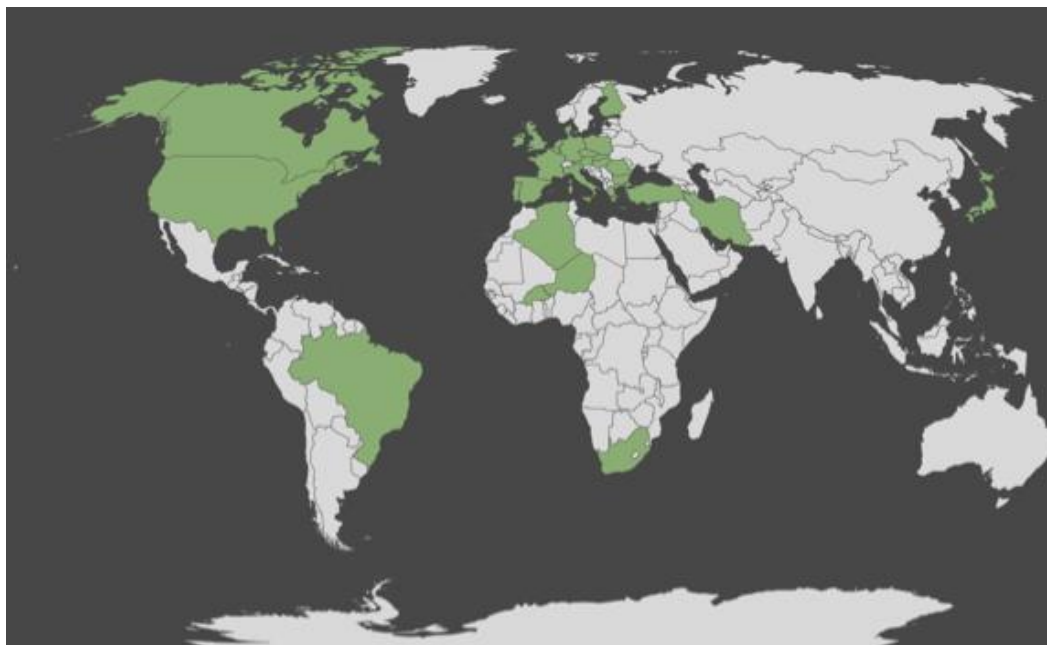


Figure 2. Scrapie distribution in all over the world in 2007 (Cosseddu et al., 2007)

As it is clear that polymorphisms at residues 136, 154, and 171 are associated with susceptibility to both, experimental and natural scrapie (Hunter, 1997). In sheep, breeding projects have been set up in a few European nations to expand the ARR allele as much as possible. To evade the negative outcomes of scrapie-safe alleles, it is indispensable to know their populace recurrence. Some programs are still working on the frequencies to get rid of the susceptible alleles. In *Table 4* we can see the genotype groups and the intensity of risk.

Prions and scrapie

Scrapie is a protein misfolding where the normal prp misfolds into abnormal prp that is extremely resistant to enzymatic breakdown within the cell and accumulates, ultimately leading to neurodegeneration. The disease is experimentally transmissible to cattle, goats, and laboratory animals *via* oral, parenteral, and intracerebral routes using homogenates of a brain or lymphoid tissues from infected animals (Pattison et al., 1961). Squirrel monkey was infected by feeding infected tissues and many other species like rats, mice, chimpanzees and many others were infected as well (de Mouton, 2007). The mode of transmission from ewe to lamb or between adults in field environments is not clear. However, oral exposure to fetal membranes or to pastures grazed by infected animals has been implicated as a possible route of vertical and horizontal transmission (Brotherston et al., 1968). Susceptibility to ovine scrapie is controlled by a combination of host genetics. During the course of a prion disease, a largely protease-resistant aggregated form of prp designated abnormal prp, accumulates mainly in the brain, and maybe the main or only constituent of the prion but in some species little or no signs of accumulation other than brain were found (de Mouton, 2007). The alteration of the normal prp into the abnormal prp is the vital route of transmission and pathogenesis of the prion disease in sheep. Transgenic studies say that abnormal prp acts as a template

on which normal prp is refolded into a nascent abnormal prp molecule through a process facilitated by another protein. Because no differences in the primary sequence were found between normal prp and abnormal prp, the two species are believed to differ only in their conformation. After normal prp is synthesized in the endoplasmic reticulum, it transits through the Golgi to the cell surface where it is bound by a glycosphosphatidyl inositol (GPI)-anchor. At or near the cell surface, normal prp is either metabolized or converted into abnormal prp (Benke et al., 2007). Normal prp seems to re-enter cells through caveolae-like domains (CLDs), a subcellular compartment defined biochemically by membranes rich in cholesterol and glycosphingolipids; this compartment also contains many GPI-anchored proteins. Polymorphisms in the prion protein gene (*PRNP*) determine the amino acid sequence of the host's prion protein and play a major role in relative susceptibility or resistance to classical scrapie. Prion protein (*PRNP*) gene is well known for affecting mammal transmissible spongiform encephalopathies (TSE) and is also reported to regulate phenotypic traits (e.g., growth traits) in healthy ruminants. As the vital control gene of fatal prion diseases or transmissible spongiform encephalopathies (TSE), the prion protein (*PRNP*) gene will always be a focus of ovine research (Houston et al., 2015; Stepanek and Horin, 2017). The *PRNP* gene encodes the prion protein (PrP), which plays a major role in the disease process (Goldmann, 2008; Houston et al., 2015). In sheep, amino acid polymorphisms at many positions (89, 94, 101, 112, 127, 128, 132, 134, 135, 136, 137, 138, 141, 143, 145, 146, 149, 151, 152, 154, 157, 159, 160, 163, 164, 167, 168, 169, 171, 172, 174, 175, 176, 180, 183, 184, 185, 189, 193, 195, 196, 199, 211, 213, 220, 224, 241) have been described (Oner et al., 2011), but the polymorphisms at codons 136, 154 and 171 have been demonstrated to be of major importance, as they modulate the susceptibility/resistance of sheep for scrapie (Cloucard et al., 1995; Hunter et al., 1996).

These polymorphisms are Alanine (A), Valine (V) or Threonine (T) at codon 136, Arginine (R) or Histidine (H) at codon 154 and Glutamine (Q), R, H or Lysine (K) at codon 171. The five most common haplotypes are ARR, ARQ, AHQ, ARH, and VRQ. New haplotypes (TRQ, ARK, VRR, AHR, VHQ, and TRR) have been reported so far. Haplotype alleles encoding three other forms of PrP (ARQ, AHQ, and ARH, where H is histidine) have intermediate associations with classical scrapie disease progression following exposure to the transmissible agent (Goldmann, 2008). Over 30 SNPs already showed that the ovine prion gene (PrP) shows an unusually high level of genetic variation (Goldmann et al., 2005). The ovine *PRNP*, mapped to chromosome 13, is a highly polymorphic gene consisting of three exons, among which only the third is translated (Lee et al., 1998). Single nucleotide polymorphisms (SNPs) leading to amino acid change in PrP were observed in over 20 codons, but most of them are rare and unrelated to disease development (Goldmann et al., 2005). It was established that polymorphisms A136V (Alanine, GCC → GTC, Valine), R154H (arginine, CGT → CAT, histidine) and Q171R (glutamine, CAG → CGG, arginine) are associated with susceptibility or resistance to scrapie (Baylis et al., 2004). Additionally, some studies reported another polymorphic variant coding for histidine at codon 171, but it is very rare (Acín et al., 2004). The combination of these polymorphisms results in the creation of 3-locus haplotypes and diploid genotypes, among which A136R154Q171 (hereafter ARQ) haplotype and AA136RR154QQ171 (hereafter ARQ/ARQ) genotype are thought to be wild-type variants. With the help of Table 4, we can see the severity or intensity of genotypes.

Table 4. According to the intensity of risk, scientists has classified the risk groups (Cosseddu et al., 2007)

Risk class	Genotype	Risk intensity
1	ARR/ARR	Sheep are highly resistant to scrapie
2	ARR/AHQ ARR/ARH ARR/ARQ	Sheep are resistant to scrapie, thus require particular attention in breeding programs
3	ARQ/ARH ARQ/AHQ AHQ/AHQ ARH/ARH AHQ/ARH ARQ/ARQ	Sheep with low genetic resistance to scrapie. Their use in breeding programs must be avoided
4	AHQ/ARH	Sheep are sensitive to scrapie
5	AHQ/VRQ ARH/VRQ ARQ/VRQ VRQ/VRQ	Sheep are highly sensitive to scrapie, thus must be castrated or culled

The rapid dissemination of scrapie over the previous limited years led to the development of a specific eradication program, based on the polymorphisms within the prion protein gene (*PRNP*). The current approach encourages the selection of animals carrying the resistant ARR/ARR genotype, while other genotypes are considered not preferable. Although the strategy seems to be working quite well, farmers are concerned whether this will affect sheep productivity and subsequently decrease net profits. Current scrapie eradication program includes genotyping and subsequent selection of animals on the ARR/ARR genotype.

Genotype and haplotype

Observing the development of *PRNP* genotype and haplotype is a powerful pointer of choice weight, which is evaluated in reference class creatures alongside ages so as to dodge predisposition, while the advancement in the common populace is assessed depending on scientific models. In countries where the scrapie incidence was statistically significant (see *Tables 5* and *6*), such as from Romania (Lacaune) the genotype frequencies are (ARR/ARR: 15.1) and (ARQ/VRQ: 12.6) (Otelea et al., 2011); from Romania (Turcana) (ARR/ARR: 14.64) and (ARQ/VRQ: 12.2) (Coşier et al., 2011); from Finland (Finnish Landrace) (ARR/ARR: 1.3) and (ARQ/VRQ: 10.3) (Hautaniemi et al., 2012); from Italy (Biellese rams) (ARQ/VRQ: 1.4) and (ARQ/VRQ: 9.9) (Acutis et al., 2004); from Slovakia (Orava) (ARR/ARR; 10.9) and (ARQ/VRQ: 9.0) (Tkáčiková et al., 2003); from Slovakia (Valachian) (ARR/ARR; 10.9) and (ARQ/VRQ: 9.0) (Tkáčiková et al., 2003); from Slovakia (Spiš) (ARR/ARR; 10.8) and (ARQ/VRQ; 8.9) (Tkáčiková et al.,

2003); from Germany (Texel) (ARR/ARR; 11.7) and (ARQ/VRQ; 7.8) (Kutzer et al., 2002); from Greece (Greek Dairy Breed) (ARR/ARR; 2.2) and (ARQ/VRQ; 5.4) (Boukouvala et al., 2018a); from Spain (Rasa Aragonesa) (ARR/ARR; 2.0) and (ARQ/VRQ; 5.0) (Acín et al., 2004); from Poland (Pomorska) (ARR/ARR; 13.3) and (ARQ/VRQ; 3.3) (Lühken et al., 2008); from Poland (Pomorska) (ARR/ARR; 13.3) and (ARQ/VRQ; 3.3) (Acín et al., 2004); from Spain (Roya Bilbilitana) (ARR/ARR; 2.0) and (ARQ/VRQ; 3.0) (Acín et al., 2004); from Poland (Kaminieniecka) (ARR/ARR; 35.3) and (ARQ/VRQ; 2.9) (Szkudlarek-Kowalczyk et al., 2010); from Germany (Nolana) (ARR/ARR; 32.4) and (ARQ/VRQ; 2.8) (Kutzer et al., 2002); from Turkey (Imroz) (ARR/ARR; 29.9) and (ARQ/VRQ; 2.7) (Oner et al., 2011); from Brazil (Santa Ines sheep) (ARR/ARR; 7.4) and (ARQ/VRQ; 2.2) (Andrade et al., 2018); from Greece (Crossbred) (ARR/ARR; 7.3) and (ARQ/VRQ; 1.9) (Kioutsoukakis et al., 2018); from Greece (Chios crossbred) (ARR/ARR; 2.7) and (ARQ/VRQ; 1.6) (Kioutsoukakis et al., 2018); from Brazil (Dorset sheep) (ARR/ARR; 11.6) and (ARQ/VRQ; 1.5) (Andrade et al., 2018); from Iran (local sheep) (ARR/ARR; 38) and (ARQ/VRQ; 1.2) (Karami et al., 2011); from Greece (chios) (ARR/ARR; 1.2) and (ARQ/VRQ; 1.2) (Kioutsoukakis et al., 2018); from Poland (Île de France) (ARR/ARR; 72.0) and (ARQ/VRQ; 1.1) (Wisniewska and Mroczkowski, 2009); from Poland (Polish Merino) (ARR/ARR; 7.1) and (ARQ/VRQ; 1.0) (Wiśniewska et al., 2006); from Turkey (Kivircik) (ARR/ARR; 1.41) and (ARQ/VRQ; 0.7) (Oner et al., 2011); from Greece (Chios) (ARR/ARR; 0.4) and (ARQ/VRQ; 0.5) (Psifidi et al., 2011); from England (15 scrapie affected flocks) (ARR/ARR; 14.8) and (ARQ/VRQ; 0.2) (Tongue et al., 2004); from Pakistan (Kajli) (ARR/ARR; 1.9) (Babar et al., 2009); from China (Gansu Alpine Merino sheep) (ARR/ARR; 20.7) (Liu et al., 2017); from the Czech Republic (Charollais) (ARR/ARR; 61.5) (Stepanek and Horin, 2017). Breeding programs planned to increase the RR171 genotype in sheep populations and eliminate affected animals, to considerably decrease the number of classical scrapie cases in America and in the European Union (Greenlee, 2019). This is cleared that these genotypes and haplotypes are the backbone in the resistance and susceptibility of scrapie, which can be low or high. From *Table 5* we can clearly say that, in Romania, Finland, Italy, Slovakia, Germany, Greece, Spain, Germany, Brazil, Iran, Poland, Turkey, Greece, England, Portugal and Hungary sheep are highly sensitive to scrapie, therefore must be eliminated or separated and in countries like Pakistan and China resistance to scrapie was observed.

The writers propose that arginine/glutamine replacement in the 171st position of the sheep *PRNP* might have affected the scrapie incubation period. In some countries the haplotype is very significant, that can be seen in *Table 6*, like the ARR in German Blackheaded Mutton; 87.0 which is the highest ARR frequency recorded and the highest VRQ frequency is recorded in Lacunae from Romania 18.9. The countries where the VRQ frequencies are found are Romania, Poland, Greece, Germany, Italy, Slovakia, Czech Republic, Finland, Germany, Austria, Spain, Turkey, Finland, Iran, Hungary; these are highly sensitive to scrapie, thus sheep must be eliminated and breeding programs must be introduced.

From these two tables and from the graphical presentation in *Figures 3* and *4*, we can see the clear difference between the countries where the scrapie is present, or the chance of scrapie is severe. The countries like China and Pakistan must take some important steps like proper breeding programs, before doing meat or any kind of trade associated with sheep with countries like Romania, Greece and the other countries found susceptible to scrapie.

Table 5. Genotypic frequencies of PRNP gene at codon 131, 154 and 171 in various breeds of sheep in major parts of the world

#	Country	Breed	N	ARR/ ARR	ARR/ ARQ	ARQ/ ARQ	ARR/ AHQ	ARQ/ AHQ	AHQ/ AHQ	ARR/ VRQ	VRQ/ VRQ	ARQ/ VRQ	Reference
1	Romania	Lacaune	159	15.1	33.3	20.1	5.0	5.7		6.3		12.6	Otelea et al. (2011)
2	Romania	Turcana	123	14.64	32.52	28.46	0.81	1.63		5.69		12.2	Coşier et al. (2011)
3	Finland	Finnish Landrace	232	1.3	15.9	68.8		0.4			0.4	10.3	Hautaniemi et al. (2012)
4	Italy	Biellese rams	1207	1.4	11.4	56.3	0.7	5.5	0.2	1.2	0.7	9.9	Acutis et al. (2004)
5	Slovakia	Orava	366	10.9	45.4	19.4	5.7	4.9	0.8	3.6	0.3	9.0	Tkáčiková et al. (2003)
6	Slovakia	Valachian	735	10.9	45.2	19.3	5.7	4.9	0.7	3.5	0.3	9.0	Tkáčiková et al. (2003)
7	Slovakia	Spiš	369	10.8	54.0	19.2	0.5	4.9	0.5	3.5	0.3	8.9	Tkáčiková et al. (2003)
8	Germany	Texel	231	11.7	19.5	25.1	0.4	2.2		6.5	0.4	7.8	Kutzer et al. (2002)
9	Greece	Greek Dairy Breed	4382	2.2	24.2	32.4		12.5	2.0	1.23	0.3	5.4	Boukouvala et al. (2018b)
10	Spain	Rasa Aragonesa	296	2.0	21.0	51.0	2.0	6.0	0.0	0.0		5.0	Acin et al. (2004)
11	Poland	Pomorska	30	13.3	36.7	16.7	6.7	6.7		3.3	3.3	3.3	Lühken et al. (2008)
12	Spain	Ojinegra	182	2.0	21	56	0.0	1	0.0	1.0		3.0	Acin et al. (2004)
13	Spain	Roya Bilbilitana	96	2.0	34	53	1.0	0	0.0	1.0		3.0	Acin et al. (2004)
14	Poland	Kaminieniecka	102	35.3	33.3	2.9				6.9		2.9	Szkudlarek-Kowalczyk et al. (2010)
15	Germany	Nolana	71	32.4	33.8	18.3				4.2		2.8	Kutzer et al. (2002)
16	Turkey	Imroz	147	29.9	33.3	19.0	6.1	5.4			0.7	2.7	Oner et al. (2011)
17	Brazil	Santa Ines sheep	94	7.4	21.3	47.8		17		1.1		2.2	Andrade et al. (2018)

#	Country	Breed	N	ARR/ ARR	ARR/ ARQ	ARQ/ ARQ	ARR/ AHQ	ARQ/ AHQ	AHQ/ AHQ	ARR/ VRQ	VRQ/ VRQ	ARQ/ VRQ	Reference
18	Greece	Crossbred	483	7.3	28.2	31.5	5.6	12.7	1.0	0.4	0.00	1.9	Kioutsoukis al. (2018)
19	Greece	Chios crossbred	633	2.7	18.9	40.8	2.0	9.9	0.8	0.6	0.3	1.6	Kioutsoukis et al. (2018)
20	Brazil	Dorset sheep	69	11.6	43.5	39.1				4.3		1.5	Andrade et al. (2018)
21	Iran	Local sheeps	250		38	43.2						1.2	Karami et al. (2011)
22	Greece	chios	340	1.2	13.2	52.9	2.1	7.1	0.6	0.3	0.00	1.2	Kioutsoukis et al. (2018)
23	Poland	Ile de France	93	72.0	6.5					17.2	3.2	1.1	Wisniewska and Mroczkowski (2009)
24	Poland	Polish Merino	98	7.1	54.1	35.7				2.0		1.0	Wiśniewska et al. (2006)
25	Turkey	Kivircik	142	1.41	24.65	30.28	1.41	7.75				0.7	One et al. (2011)
26	Greece	Chios	1013	0.4	11.4	56.0	0.5	15.0	0.1			0.5	Psifidi et al. (2011)
27	England	15 scrapie affected flocks	3732	14.8	30.7	15.7	7.8	8.2	1.5	7.6	0.8	0.2	Tongue et al. (2004)
28	Portugal	Merino Branco	62	0.194	0.387	0.306	0.065	0.016	0.00	0.016		0.00	Mesquita et al. (2010)
29	Portugal	Saloia	52	0.096	0.231	0.442	0.038	0.135	0.00	0.019		0.019	Mesquita et al. (2010)
30	Portugal	Serra da Estrela	69	0.174	0.420	0.304	0.014	0.014	0.00	0.014		0.029	Mesquita et al. (2010)
31	Portugal	Bordaleira entre Douro e Minho	64	0.078	0.250	0.469	0.047	0.047	0.0	0.016		0.047	Mesquita et al. (2010)
32	Portugal	Churra Badana	58	0.052	0.345	0.517	0.017	0.052	0.0	0.00		0.00	Mesquita et al. (2010)
33	Portugal	Churra Galega Mirandesa	71	0.014	0.253	0.549	0.0	0.099	0.014	0.00		0.056	Mesquita et al. (2010)
34	Portugal	Churra Mondegueira	19	0.053	0.105	0.737	0.0	0.00	0.00	0.00		0.00	Mesquita et al. (2010)
35	Portugal	Merino da Beira-Baixa	65	0.092	0.231	0.523	0.0	0.31	0.00	0.031		0.077	Mesquita et al. (2010)

#	Country	Breed	N	ARR/ ARR	ARR/ ARQ	ARQ/ ARQ	ARR/ AHQ	ARQ/ AHQ	AHQ/ AHQ	ARR/ VRQ	VRQ/ VRQ	ARQ/ VRQ	Reference
36	Hungary	Hungarian Tsigai	392	0.27	0.4	0.2	0.06	0.02	0.0	0.02	0.06	0.06	Mari (2016)
37	Greece	Karagouniko	100		14.5	32		6.0			7.9		Billinis et al. (2004)
38	Austria	Carynthian sheep	24	4.2	37.5	41.6		4.2			4.2		Sipos et al. (2002)
39	Turkey	Chios	124	15.32	22.58	20.16	1.61	7.26	1.61		0.8		Oner et al. (2011)
40	Poland	Olkuska	174	35.1	60.9	4.0							Kaczor et al. (2011)
41	Poland	Gorska	31	12.9	51.6	22.6	3.2	9.7					Lühken et al. (2008)
42	Poland	Wrzosowka	31	6.5	48.4	9.7	19.3	12.9	3.2				Lühken, Lipsky et al. (2008)
43	Finland	Kainuu	48		16.7	83.3							Hautaniemi et al. (2012)
44	Germany	Suffolk	87	14.9	20.7	54.0	1.1	1.1					Kutzer et al. (2002)
45	Pakistan	Awassi	21		4.8	92.2							Babar et al. (2009)
46	Pakistan	Buchi	35			100							Babar et al. (2009)
47	Pakistan	Hissardale	20	5		70							Babaret al. (2009)
48	Pakistan	Kajli	52	1.9	9.6	84.6							Babaret al. (2009)
49	Pakistan	Lohi	50		10	88							Babaret al. (2009)
50	Pakistan	Pak-Karakul	19		36.8	63.2							Babaret al. (2009)
51	Pakistan	Sipli	41			65.9							Babaret al. (2009)
52	Pakistan	Thalli	40		25	100							Babaret al. (2009)
53	Pakistan	kachi	30			100							Babaret al. (2009)
54	China	Gansu Alpine Merino sheep	111	20.7	27	46							Liu et al. (2017)

#	Country	Breed	N	ARR/ ARR	ARR/ ARQ	ARQ/ ARQ	ARR/ AHQ	ARQ/ AHQ	AHQ/ AHQ	ARR/ VRQ	VRQ/ VRQ	ARQ/ VRQ	Reference
55	Algeria	Barbarine	20		20	40							Djaout et al. (2018)
56	Algeria	Berbere	20	5	20	20							Djaout et al. (2018)
57	Algeria	Hamra	27		19	11							Djaout et al. (2018)
58	Algeria	Ouled Djellal	35	8	17	11							Djaout et al. (2018)
59	Algeria	Rembi	40	8	20	18							Djaout et al. (2018)
60	Algeria	Sidaou	30		3	23							Djaout et al. (2018)
61	Algeria	Taadmit	10	20	10	10							Djaout et al. (2018)
62	Algeria	Tazegzawt	31		10	23							Djaout et al. (2018)
63	Czech Republic	Berrichone du Cher	445	54.6									Stepanek and Horin (2017)
64	Czech Republic	Charollais	3219	61.5									Stepanek and Horin (2017)
65	Czech Republic	East Friesian sheep	1864	56.5									Stepanek and Horin (2017)
66	Czech Republic	German Blackheaded Mutton	628	75.3									Stepanek and Horin (2017)
67	Czech Republic	Kent Romney)	5995	38.0									Stepanek and Horin (2017)
68	Czech Republic	Merinolandschaf	2057	33.1									Stepanek and Horin (2017)
69	Czech Republic	Oxford Down	1044	59.5									Stepanek and Horin (2017)
70	Czech Republic	Romanov sheep	3281	44.2									Stepanek and Horin (2017)
71	Czech Republic	Sumavka	3358	23.1									Stepanek and Horin (2017)
72	Czech Republic	Suffolk	12987	73.9									Stepanek and Horin (2017)

#	Country	Breed	N	ARR/ ARR	ARR/ ARQ	ARQ/ ARQ	ARR/ AHQ	ARQ/ AHQ	AHQ/ AHQ	ARR/ VRQ	VRQ/ VRQ	ARQ/ VRQ	Reference
73	Czech Republic	Texel	3142	72.7									Stepanek and Horin (2017)
74	Czech Republic	Valachian sheep	1301	55.9									Stepanek and Horin (2017)
75	Czech Republic	Zwartbles	1791	39.6									Stepanek and Horin (2017)
76	West Africa	Burkina-Sahel	46		6.5	80.4	2.2	10.9					Traoré et al. (2012)
77	West Africa	Djallonké	50			86.0		12.0	2.0				Traoré et al. (2012)
78	West Africa	Mossi	46		6.5	76.1		15.2	2.2				Traoré et al. (2012)
79	West Africa	Touareg	20			40.0		55.0	5.0				Traoré et al. (2012)
80	Finland	Grey race sheep	48		16.7	83.3							Hautaniemi et al. (2012)
81	Finland	Aland sheep	56		23.2	48.2		19.6	8.9				Hautaniemi et al. (2012)
82	Finland	Taxel	71	1.4	31.0	33.8		8.5					Hautaniemi et al. (2012)
83	America	Suffolk	128	36.72	43.75	17.19							DeSilva et al. (2003)
84	America	Montadale	47	17.02	23.40	19.15							DeSilva et al. (2003)
85	America	Hampshire	91	20.88	52.75	26.37							DeSilva et al. (2003)
86	America	Dorset	62	9.68	38.71	38.71							DeSilva et al. (2003)
87	Austria	Tyrolean mountain	35	2.9	40.0	40.0	5.7	11.4					Sipos et al. (2002)
88	Austria	Forest sheep	26	111.5	15.4	57.7		11.5					Sipos et al. (2002)
89	Austria	Tyrolean stone sheep	27		29.6	40.7		22.2					Sipos et al. (2002)

Table 6. Haplotypic frequencies of PRNP gene at codons 136, 154 and 171: alanine (A), arginine (R), histidine (H), glutamine (Q) and valine (V) in various breeds of sheep in major parts of the world

#	Country	Breed	N	ARR	ARQ	AHQ	VRQ	Reference
1	Romania	Lacaune	159	15.1	60.4	5.0	18.9	Otelea et al. (2011)
2	Poland	Ile de France	93	83.8	3.8		12.4	Wisniewska and Mroczkowski (2009)
3	Greece	Greek Dairy Breed	4382	0.1	9.4	10	11.9	Boukouvala et al. (2018b)
4	Romania	Turcana	123	34.1	53.7	1.2	8.9	Coşier et al. (2011)
5	Poland	Pomorska	30	40.0	40.0	6.7	8.3	Wiśniewska et al. (2006)
6	Germany	Texel	231	28.8	44.4	1.9	8.2	Kutzer et al. (2002)
7	Italy	Biellese rams	2414	8.3	74.4	3.8	6.8	Acutis et al. (2004)
8	Slovakia	Valachian	735	38.4	48.7	6.0	6.5	Tkáčiková et al. (2003)
9	Czech Republic	Valachian sheep	1301	74.3			5.7	Stepanek and Horin (2017)
10	Finland	Finnish Landrace	464	9.5	83.4	0.2	5.6	Hautaniemiet al. (2012)
11	Germany	Nolana	71	52.8	36.6		5.6	Kutzer et al. (2002)
12	Poland	Kaminieniecka	102	63.2	21.6		5.4	Szkudlarek-Kowalczyk et al. (2010)
13	Poland	Zelanienska	31	46.8	43.6	4.8	4.8	Lühken et al. (2008)
14	Austria	Carynthian sheep	24	23.0	64.6	4.2	4.2	Sipos et al. (2002)
15	Spain	Rasa Aragonesa	296	15.0	70.9	4.8	2.9	Acin et al. (2004)
16	Spain	Roya Bilbilitana	96	21.4	72.4	1	2.6	Acin et al. (2004)
17	Czech Republic	Romanov sheep	3281	66.3			2.5	Stepanek and Horin (2017)
18	Turkey	Imroz	147	50.0	40.1	5.8	2.4	Oner et al. (2011)
19	Greece	Chios crossbred	633	15.0	63.4	7.7	1.7	Kioutsioukis et al. (2018)
20	Czech Republic	Zwartbles	1791	63.7			1.6	Stepanek and Horin (2017)
21	Greece	Crossbred	483	25.7	55.9	11.3	1.5	Kioutsioukis et al. (2018)
22	Poland	Polish Merino	98	35.2	63.3		1.5	Wiśniewska et al. (2006)
23	Finland	Taxel	144	17.6	64.1	4.2	1.4	Hautaniemiet al. (2012)
24	Czech Republic	Charollais	3219	79.6			1.3	Stepanek and Horin (2017)
25	Turkey	Kivircik	142	30.64	39.52	6.83	0.8	Oner et al. (2011)
26	Greece	Chios	340	10	71.5	5.8	0.7	Kioutsioukis et al. (2018)

#	Country	Breed	N	ARR	ARQ	AHQ	VRQ	Reference
27	Iran	Local sheeps	250		67.8		0.6	Karami et al. (2011)
28	Czech Republic	East Friesian sheep	1864	76.1			0.5	Stepanek and Horin (2017)
29	Czech Republic	Sumavka	3358	46.1			0.5	Stepanek and Horin (2017)
30	Greece	Chios	1013	6.9	76.1	8.2	0.4	Psifidi et al. (2011)
31	Turkey	Chios	124	17.25	56.69	5.63	0.4	Oner et al. (2011)
32	Czech Republic	Berrichone du Cher	445	75.5			0.4	Stepanek and Horin (2017)
33	Spain	Ojinegra	182	14.6	73.9	3	0.3	Acín et al. (2004)
34	Greece	Random breeds	5815	47.7	44.9	3.9	0.3	Boukouvala et al. (2018b)
35	Czech Republic	Texel	3142	85.9			0.2	Stepanek and Horin (2017)
36	Czech Republic	Suffolk	12987	86.28			0.1	Stepanek and Horin (2017)
37	Czech Republic	Merinolandschaf	2057	59.3			0.1	Stepanek and Horin (2017)
38	Czech Republic	Kent Romney)	5995	63.5			0.1	Stepanek and Horin (2017)
39	Hungary	Hungarian Tsigai	569	0.5	0.4	0.05	0.01	Mari (2016)
40	Czech Republic	German Blackheaded Mutton	628	87.0			0.0	Stepanek and Horin (2017)
41	Czech Republic	Oxford Down	1044	79.2			0.0	Stepanek and Horin (2017)
42	Finland	Grey race sheep	96	8.3	91.7	0	0	Hautaniemiet al. (2012)
43	Finland	Aland sheep	112	11.6	69.6	18.8	0	Hautaniemiet al. (2012)
44	Poland	Olkuska	174	65.5	34.5			Kaczor et al. (2011)
45	Poland	Polish Mountain	31	40.3	53.2	6.5		Wiśniewska et al. (2006)
46	Poland	Wrzosowka	31	41.9	38.7	19.4		Lühken et al. (2008)
47	Finland	Kainuu	48	8.3	91.7			Hautaniemi et al. (2012)
48	Greece	Karagouniko	100	28.5	66.0	3.0		Billinis et al. (2004)
49	Germany	Suffolk	87	27.0	67.2	1.1		Kutzer et al. (2002)
50	China	Xinjiang Sheeps	222	9.0	75.2	2.3		Lan et al. (2006)
51	Algeria	Barbarine	20	15	65			Djaout et al. (2018)
52	Algeria	Berbere	20	18	48	3		Djaout et al. (2018)
53	Algeria	Hamra	27	11	41	7		Djaout et al. (2018)
54	Algeria	Ouled Djellal	35	26	31	3		Djaout et al. (2018)

#	Country	Breed	N	ARR	ARQ	AHQ	VRQ	Reference
55	Algeria	Rembi	40	24	43	3		Djaout et al. (2018)
56	Algeria	Sidaou	30	8	45	2		Djaout et al. (2018)
57	Algeria	Taadmit	10	30	30	10		Djaout et al. (2018)
58	Algeria	Tazegzawt	31	8	47	3		Djaout et al. (2018)
59	Burkina Faso	Burkina-Sahel	46	4.4	89.1	6.5		Traoré et al. (2012)
60	Burkina Faso	Djallonké	50		92.0	8.0		Traoré et al. (2012)
61	Burkina Faso	Mossi	46	3.2	87.0	9.8		Traoré et al. (2012)
62	Niger	Touareg	20		67.5	32.5		Traoré et al. (2012)
63	Austria	Tyrolean mountain	35	25.8	65.7	8.6		Sipos et al. (2002)
64	Austria	Forest sheep	26	19.2	71.2	5.8		Sipos et al. (2002)
65	Austria	Tyrolean stone sheep	27	14.8	70.3	11.1		Sipos et al. (2002)
66	China	Lanzhou large-tailed sheep	30	0	26	0		Lan et al. (2014)
67	China	Mongol sheep	30	0	26	0		Lan et al. (2014)
68	China	Tan sheep	30	0	25	0		Lan et al. (2014)
69	China	Gaoyuan sheep	30	0	32	0		Lan et al. (2014)
70	China	Guide fur sheep	30	0	28	0		Lan et al. (2014)
71	China	Oula sheep	30	0	30	0		Lan et al. (2014)
72	China	Lowland sheep	30	0	28	0		Lan et al. (2014)
73	China	Sishui fur sheep	30	0	21	0		Lan et al. (2014)
74	China	Small-tailed Han sheep	30	0	22	0		Lan et al. (2014)
75	China	Hu sheep	30	2	25	0		Lan et al. (2014)
76	China	Tong sheep	30	1	25	0		Lan et al. (2014)
77	China	Duolang sheep	30	1	27	0		Lan et al. (2014)
78	China	Diqing sheep	30	2	30	1		Lan et al. (2014)
79	China	Tengchong sheep	30	1	12	17		Lan et al. (2014)
80	China	Zhaotong sheep	30	1	7	22		Lan et al. (2014)
81	China	Tibetan sheep	30	3	27	0		Lan et al. (2014)

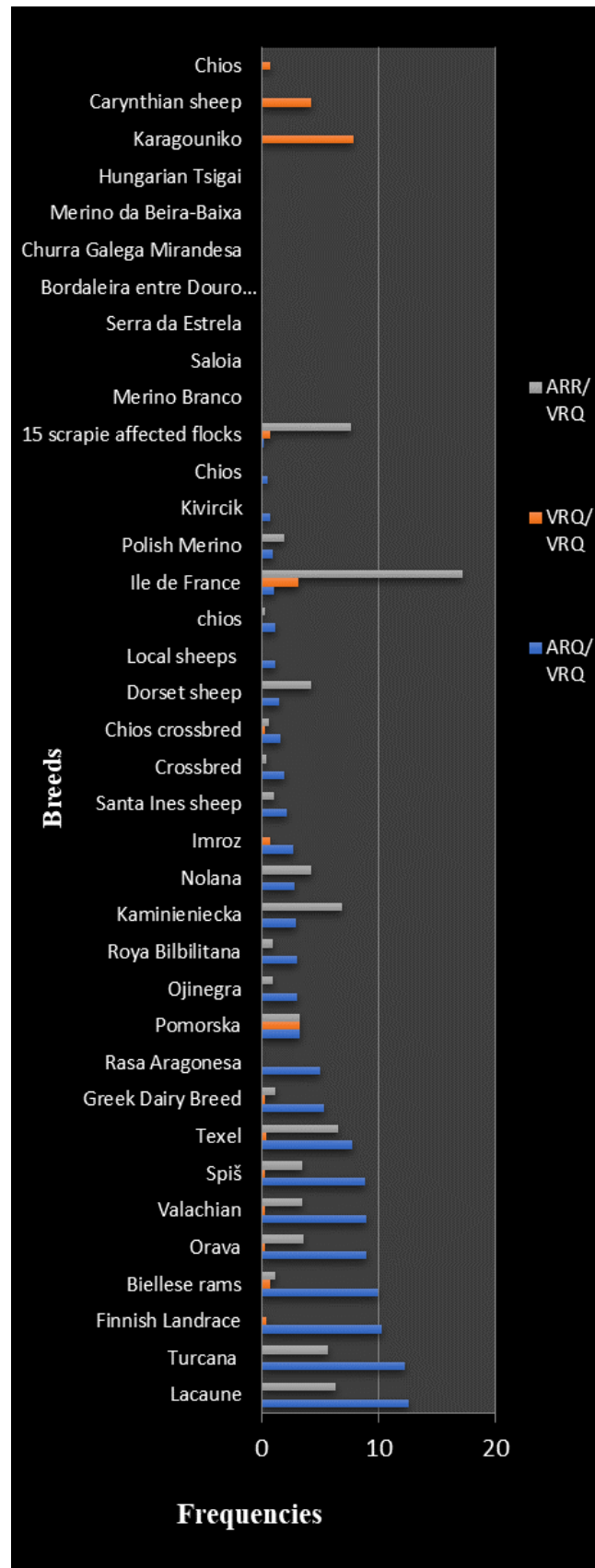


Figure 3. Genotype frequencies of PRNP gene at codons 136, 154 and 171. (Constructed from Table 5)

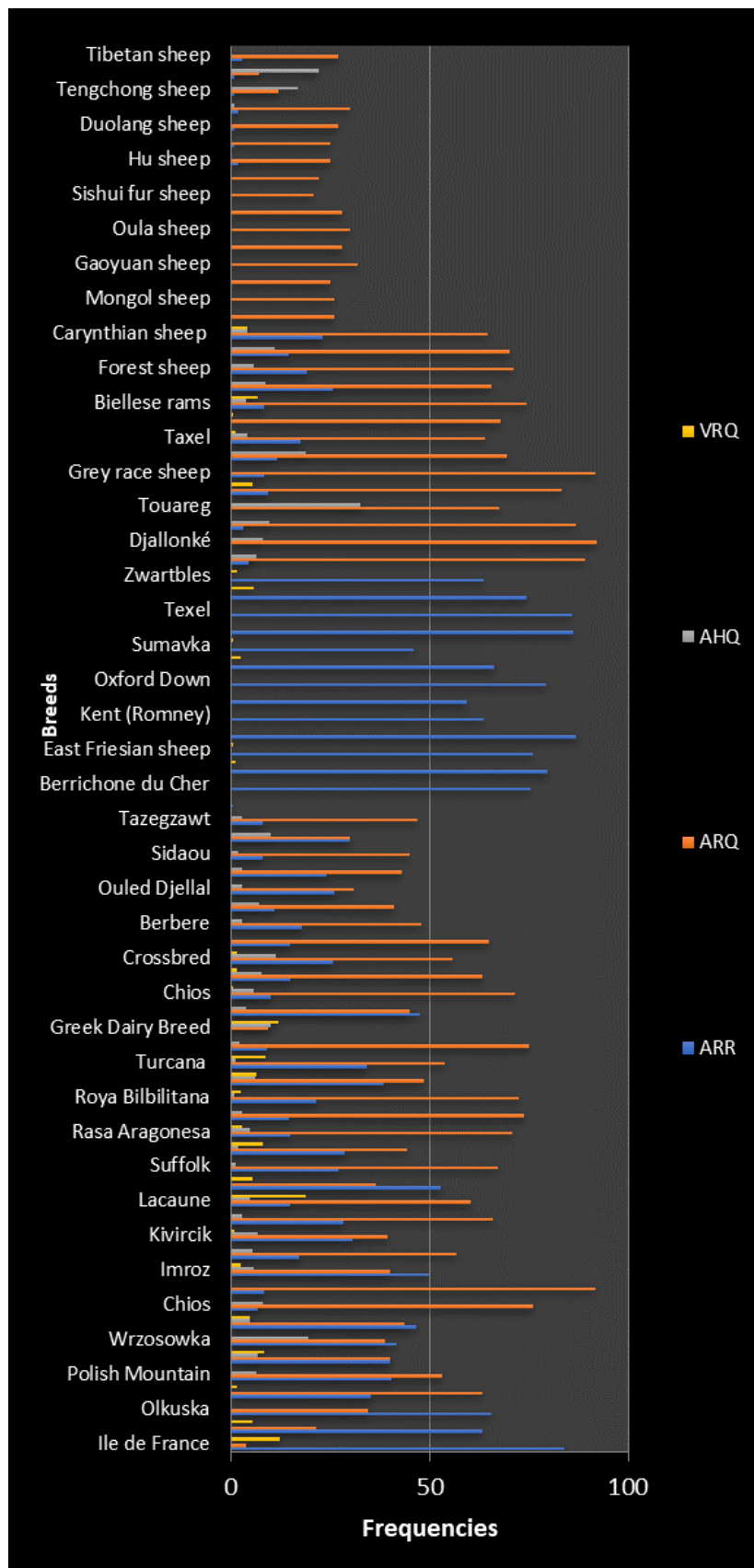


Figure 4. Haplotype frequencies of PRNP gene at codons 136, 154 and 171. (Constructed from Table 6)

Summary

The purpose of this review is to highlight the countries where scrapie is causing problems and provide some information about the countries where scrapie is not present but there is possibility of its occurrence because scrapie is a prion disease and scientists still do not know how to cure the disease. It is very difficult to get rid of this if once entered in a country, the complete rid is only possible with the help of special breeding programs. Almost every country is working hard on breeding programs to get rid of scrapie, because these breeding programs showed positive signs in countries like Australia and New Zealand which are now scrapie free. But actual facts of scrapie in many parts of the world remain unknown due to the unsatisfactory passive surveillance system, because of which we cannot get consistent results or conclusion. As already described scrapie or any other prion disease do not have any treatment, so the only way to protect the animals is taking proper precautionary measures. The causative agent of the scrapie has still not been fully identified. All routes of transmission and their relative importance are still unknown. Animal health is directly related to humans, for the safety of humans, we must try to find some serious solutions for the well-being of animals. Like in humans, for the early diagnosis of sCJD many scientists have preferred the polysomnogram to detect earlier changes in sCJD patients may be praiseworthy. In humans, the accessory examinations of magnetic resonance imaging (MRI), electroencephalography (EEG), combined with this evidence and clinical symptom; scientists made a clinical diagnosis of sCJD. Though various drugs have been tried *in vitro* and/or *in vivo*, only four drugs have been studied in larger-scale observational or placebo-control trials: flupirtine, quinacrine, pentosan polysulfate (PPS), and doxycycline (Trevitt and Collinge, 2006). In sheep scientists must try some special drugs, any kind of hormonal changes which can prolong the survival or try to find any other way by which animal can show the clinical signs on early stage so that they can be culled or separated from healthy animals. Continuous study and research programs are needed to clear risk factor especially those affecting for human health. Yet, we do not have effective results which can lead us to the solution. The only possible solution is to carry out proper breeding programs. Continuity investigation is needed on this research for the well-being of the mankind and for the well-being of the animals. Further studies are needed to clarify the transmission of scrapie to humans.

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