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## Prion Protein Gene Polymorphisms in the Alpha-Helical Region in Feral Pigs From Texas

Maram Alsmady  
Fort Hays State University, m\_alsmady@mail.fhsu.edu

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PRION PROTEIN GENE POLYMORPHISMS IN THE  
ALPHA-HELICAL REGION IN FERAL  
PIGS FROM TEXAS

being

A Thesis Presented to the Graduate Faculty  
of the Fort Hays State University in  
Partial Fulfillment of the Requirements for  
the Degree of Master of Science

by

Maram Alsmady

B.S., Jordan University of Science and Technology

M.S., Jordan University of Science and Technology

Date May 8, 2019

Approved   
Major Professor

Approved   
Chair, Graduate Council

This thesis for  
The Master of Science Degree

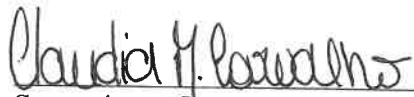
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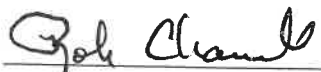
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## ABSTRACT

Prion diseases are a group of infectious, incurable, fatal neurodegenerative disorders, including scrapie in sheep, bovine spongiform encephalopathy in cows, chronic wasting disease in deer, and Creutzfeldt-Jakob disease (CJD) in humans. A key event in prion disease is the conformational transition of the cellular prion protein (PrP<sup>C</sup>) into the pathogenic isoform (PrP<sup>SC</sup>). Prion disease occurrence depends mainly on the interaction between the host prion protein (PrP<sup>C</sup>) and the prion strain (PrP<sup>SC</sup>). It was hypothesized that prion gene polymorphisms correlate with an organism's susceptibility to prion disease, which may be related to the overall stability of the  $\alpha$ -helical domain of the protein. Prion gene polymorphisms are also related to the species barrier between different mammalian species. The closer the three-dimensional conformation of the prion proteins in donor and recipient animals, the easier it is to transmit prion diseases between the two. Interestingly, some animal species are considered resistant to prion diseases, such as pigs, rabbits, and dogs, since no single case of naturally-occurring disease has been reported in them. In this research, I looked for polymorphisms in the PrP<sup>C</sup> gene by comparing wild pig sequences to each other, as well as other susceptible and resistant species. There are several regions in the nucleotide sequence in the PrP<sup>C</sup> gene of all pigs that are highly conserved. A key polymorphism seems to reside at position 224. This polymorphism might be used as a prediction tool of the animal susceptibility for prion diseases using the amino acid sequence.

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# TABLE OF CONTENTS

	Page
ABSTRACT .....	i
ACKNOWLEDGMENTS .....	ii
TABLE OF CONTENTS .....	iii
LIST OF FIGURES .....	v
LIST OF APPENDIXES .....	viii
PREFACE .....	ix
INTRODUCTION .....	1
Prion Diseases.....	1
History of Prion Disease.....	1
Causative Agent of Prion Disease.....	2
Pathogenesis of Prion Disease.....	5
Signs and Symptoms of Prion Disease.....	7
Species Barriers of Prion Disease.....	8
MATERIALS AND METHODS.....	12
Sample Collection.....	12
Genomic DNA Extraction.....	12
Polymerase Chain Reaction (PCR).....	12
Agarose Gel Electrophoresis, PCR Purification, and Sequencing.....	13
Analysis.....	13
RESULTS.....	15
Prion Protein Gene Detection by PCR.....	15

Multiple Alignment of the Amino Acid Sequence.....	15
Phylogenetic Tree.....	16
DISCUSSION.....	17
CONCLUSION.....	20
LITERATURE CITED.....	34

## LIST OF FIGURES

Figure		Page
1	<p>Examination of child infected with Kuru in Papua New Guinea. Children and women were more likely to be affected with kuru, as specific kin had specific rights to certain body parts .....</p>	21
2	<p>Difference in <math>\beta</math>-sheets content in PrP<sup>C</sup> and PrP<sup>SC</sup>. This change results in different physicochemical properties such as solubility, structure, and stability .....</p>	22
3	<p>Mouse prion protein PrP (residues 23–231). The C-terminal structural region with a GPI anchor and non-structured N-terminal region that binds copper ions.....</p>	23
4	<p>Figure 4: Human prion protein, N-terminal and C-terminal regions. The N-terminal domain of PrP is non-structured region containing five octapeptide repeats, the hydrophobic region is blue color. The C-terminal globular domain contains two <math>\beta</math>-sheets and three <math>\alpha</math>-helices. The C-terminal region contains single disulfide bridge, two N-glycosylation sites, and glycoposphatidylinositol-anchor.....</p>	24
5	<p>Histopathological lesion, vacuolation of the central nervous system in a classic CJD patient .....</p>	25



6	Species barriers to prion were identified by running experimental inoculation of different prion strains into different mammalian species. For example, mink prion can be transmitted to hamsters but not mice. And mice are highly resistant to hamster scrapie.....	25
7	Transgenic mice expressing chimeric mouse/hamster prion protein showed a region of prion protein that has a major role in the transmission of hamster scrapie to mice. When amino acid residues 108 to 189 were derived from hamster, mice were susceptible to hamster prion disease. However, when the sequence was derived from mouse prion protein, mice were resistant to hamster scrapie infection ...	26
8	Agarose gel electrophoresis of PCR products of PrP <sup>C</sup> gene (774bp) for 15 feral pigs.....	27
9	Multiple amino acid sequence alignment for prion protein of resistant animals, located above the line and susceptible animals are located below the line. Alignment was obtained using Unipro UGENE .....	28
10	Neighbor-joining tree for amino acid sequences of different mammalian species including feral pig samples, produced with MEGAX software.....	29
11	Four octa repeats (PHGGGWGQ) in prion proteins for different species, some organisms have single amino acid deletion mutation such as human and rabbits. Alignment was obtained using Unipro UGENE.....	30

12	Prion protein polymorphisms, at position 107 all mammals have shown serine except canines, which have asparagine. Also, at position 163 canines have aspartic acid instead of asparagine. At position 181 canines have arginine instead of histidine. Canines also have valine at position 219, where other mammals have isoleucine. Alignment was produced using Unipro UGENE.....	31
13	Three-dimensional structure of pigs' prion protein. This figure shows non neighbor amino acids, for example, amino acids 149, 208, and 212 are very close to each other and their interaction affects the overall structure. This is similar for amino acids 119, 120, 156, and 193. Image from SWISS-MODEL website .....	33

## LIST OF APPENDIXES

	Page
1. Amino acid sequences of different mammalian species .....	42
2. An Institutional Animal Care and Use Committee (IACUC).....	52

## PREFACE

This thesis follows the style of American Society for Microbiology.

## INTRODUCTION

### **Prion Diseases**

Prion diseases are group of rare, infectious, incurable, fatal neurodegenerative conditions (Pastore and Zagari 2007), including scrapie in sheep, bovine spongiform encephalopathy (BSE) in cow, chronic wasting disease (CWD) in deer (Bosque 2002), kuru, Creutzfeldt-Jakob, Gerstmann–Sträussler–Scheinker diseases, and fatal familial insomnia in humans (Imran and Mahmood 2011). Creutzfeldt-Jakob disease (CJD) is the most common form among human prion diseases (Knight 2017). The prevalence of human prion diseases is 1–2 persons per million worldwide each year (Chen and Dong 2016).

### **History of Prion Diseases**

Prion diseases have been known for more than 200 years. In 1732, scrapie was first reported in Spanish merino sheep (Liberski 2012). This happened when Spanish shepherds observed their sheep scraping themselves against the wall, a condition that was later called scrapie (Zabel and Reid 2015). Early in the 20th century, Hans Gerhard Creutzfeldt in 1913 (Conti1 2016) and Alfons Maria Jakob in 1921 identified a condition similar to scrapie, currently known as Creutzfeldt-Jakob disease, and was later classified with the transmissible spongiform encephalopathies (TSEs) (Gambetti et al, 2003).

In humans, kuru is a prion disease that attacked the Fore tribe in the Eastern Highlands of Papua New Guinea (Figure 1), and to a lesser degree the surrounding tribes that had close relationships such as marriage (Alpers 2008). The word kuru means “to shake from fear”, it is derived from a Fore language phrase that describes the symptoms associated with this condition (Hornabrook 1975). Kuru was first recognized in the

1950s, however, the beginning of the first case was dated to the 1920s (Collinge et al, 2008). The cause of kuru and the reason why this condition was associated with these tribes is due to their funeral ritual practices. They practiced endocannibalism as a part of their funeral rituals. Once a family member died, the remaining family members would cook and eat them (Alpers 2008). Kuru was epidemic in the period between 1957-1961, with about 1000 deaths. Luckily, kuru has mostly disappeared after the cessation of endocannibalism in the 1950s (Alpers 2008). However, kuru has a very long incubation period that exceeds 50 years and cases could still arise (Alpers 2008).

In United Kingdom in 1986 the first bovine spongiform encephalopathy (BSE) was detected (Edinburgh 2017; Monaco 2013). BSE is thought to have resulted from feeding cattle meat and bone meal from scrapie-infected sheep (Orge et al, 2015). In 1993, one hundred twenty thousand cattle were diagnosed with BSE, two years later three human victims were infected with variant Creutzfeldt-Jakob disease (vCJD). In 1996, ten cases of vCJD in people were reported, at this point, the United Kingdom government admitted that BSE can be transmitted to humans, and this outbreak forced the government to destroy 4.5 million cattle (Edinburgh 2017; Monaco 2013).

### **Causative Agent of Prion Diseases**

Prions are proteinaceous infectious particles that are free of nucleic acid and resistant to inactivation by most methods that destroy or change nucleic acids (Zabel and Reid 2015). The causative agent is the misfolded form of the normal cellular prion protein PrP<sup>C</sup> into a pathogenic isoform PrP<sup>SC</sup> (Figure 2). PrP<sup>C</sup> contains 40%  $\alpha$ -helices and 3%  $\beta$ -sheets, however, PrP<sup>SC</sup> consists of 30%  $\alpha$ -helices and 40%  $\beta$ -sheets (Figure 2) (Aguzzi and Calella 2009).

Unfortunately, the precise function of PrP<sup>C</sup> is unknown; however, scientists have suggested several important roles. These include copper transportation into cells, protection of nerve cells from injury, communication between neurons (Wulf et al, 2017), and memory functions (Sakudo et al, 2011).

PrP<sup>C</sup> is a host-encoded glycoprotein that is anchored to the plasma membrane *via* a glycosylphosphatidylinositol (GPI) anchor (Pastore and Zagari 2007). The human PrP gene encodes a protein composed of 253 amino acids (Pastore and Zagari 2007). Twenty-two amino acid residues are lost post-processing, resulting in 231 residues total (Rodriguez et al, 2017). Prion proteins are highly conserved among mammals (Figure 3) (Wulf et al, 2017). Human PrP<sup>C</sup> has 94.9% identity with sheep PrP<sup>C</sup>, 99.2% with chimpanzee PrP<sup>C</sup>, and 92.8% with cow PrP<sup>C</sup> (Pastore and Zagari 2007).

PrP is divided into two distinct regions with different structural and functional properties: the flexible N-terminal region up to residue ~120 and the structural C-terminal region. The N-terminal region contains variable numbers of octapeptide PHGGSWGQ repeats that differ among organisms, followed by a glycine-alanine rich segment (Rodriguez et al, 2017). The C-terminal area consists of a globular fold of three  $\alpha$ -helices and two  $\beta$ - sheets. It starts with a short  $\beta$ -strand that leads to the first  $\alpha$ -helix, then another short  $\beta$ -strand, which is followed by a short turn that leads to the remaining  $\alpha$ -helices (Rodriguez et al, 2017). The C-terminal portion of the prion protein is attached to the membrane through the GPI anchor (Figure 4) (Rodriguez et al, 2017).

The primary structures of PrP<sup>C</sup> and PrP<sup>SC</sup> are the same, they have the same amino acid sequence. However, they have different secondary and tertiary structures, which allows them to possess different physicochemical properties and functions (Yi et al, 2018). PrP<sup>SC</sup> is highly resistant to inactivation, for instance, sterilization methods that are used for bacteria and viruses such as alcohol, autoclaving at 121°C for 20 minutes and  $\gamma$ -ray irradiation are not effective for prion inactivation (Sakudo et al, 2011). Prion inactivation requires special treatment, such as autoclaving under severe conditions of 134°C for 18 minutes, NaOH (1 N, 20°C, 1 h), sodium dodecyl sulfate SDS (30%, 100°C, 10 min), or NaOCl (20000 ppm, 20°C, 1 min) is recommended (Sakudo et al, 2011).

The misfolded form also shows resistance to proteolytic enzymes, exposing PrP<sup>SC</sup> to proteolytic enzymes will result in N terminal digestion up to residues 81-95, depending on the strain (Kupfer et al, 2009). Increased resistance to proteolytic enzymes tends to increase the aggregation ability of PrP<sup>SC</sup> segments (Kupfer et al, 2009).

The formation and propagation of the abnormal prion protein is thought to be through the misfolding of the cellular prion protein, followed by aggregation and deposition of misfolded form within the cells. The aggregated protein recruits and converts further cellular prion protein. The misfolded protein builds up in the brain, damaging the neurons, and producing microscopic sponge-like vacuoles in the brain. (Kupfer et al, 2009). The actual process of how this misfolding and recruiting occurs is still unknown (Pezza and Serio 2007).

Prion diseases can be classified into familial, sporadic, or acquisition by infection (Gambetti et al, 2003). All forms share the pathogenic mechanism in which the cellular



prion protein converts into the misfolded form (Gambetti et al, 2003). The cause of sporadic Creutzfeldt-Jakob disease (sCJD) is unknown, however the familial forms such as fatal familial insomnia (FFI) and Gerstmann–Sträussler–Scheinker syndrome (GSS) are associated with mutations of the prion protein gene (*PRNP*), which encodes PrP<sup>C</sup> (Gambetti et al, 2003). There are more than twenty mutations of *PRNP* that are linked to prion disease. For example, a known polymorphism is located at codon 129, either encodes for methionine or valine, and affects the susceptibility to sporadic or acquired TSEs, as well as the age of onset of the disease. Therefore, amino acid mutations might alter the stability of PrP<sup>C</sup>, as well as its ability to interact and form amyloid plaques (Kupfer et al, 2009). The acquired form of prion disease is caused by the transmission of infectious prion particles as in case of kuru, BSE, chronic wasting disease (CWD), and scrapie (Gambetti et al, 2003). In humans 85–90 % of CJD cases occur sporadically, 10 % of CJD cases are familial, and the acquired CJD is observed in 2–5 % of the cases (Chen and Dong 2016).

### **Pathogenesis of Prion Diseases**

Prion diseases are devastating and untreatable conditions. The exact pathogenesis process and the infectious nature of prion agents are not yet completely understood. Protein Misfolding Cyclic Amplification (PMCA) and the Real Time Quaking-Induced Conversion (RT-QuIC) enable in vitro amplification and assessment of prion agents (Schmitz et al, 2016). The PMCA method enables in vitro amplification of PrP<sup>SC</sup> from a small quantity of PrP<sup>SC</sup> as a seed by sequential cycles of incubation and sonication (Sakudo et al, 2011). PMCA is considered the most sensitive method for detecting PrP<sup>SC</sup> reported so far (Sakudo et al, 2011).

A key event in prion disease pathogenesis is the conversion of normal cellular prion protein into partially protease-resistant prion protein (White and Mallucci 2009). However, the mechanism by which prions use to reach the central nervous system and replicate is still not well understood.

The nasal cavity, oral cavity, and gastrointestinal tract are all-natural routes for prion exposure. The immune system always works to eliminate any detectable microbes such as bacteria or viruses, however, in prion disease it seems that the immune system is playing a role in the pathogenesis and the transmission of the misfolded protein into the nervous system (Zabel and Avery 2015).

Following oral ingestion of prion containing food, prion infectious agents accumulate in the gut-associated lymphoid tissues (GALT), such as the Peyer's patches. This occurs by the help of specialized epithelial cell called microfold (M) cells, that are found in the mucosal immune system of the gastrointestinal tract, tonsils, upper and lower airways, and the conjunctiva of the eye (Gebert and Pabst 1999). M cells act as an antigen sampling system, which samples the lumen content and transports the antigen from the lumen to the cells of the immune system (Takakura et al, 2011). As any other pathogen, prions are transported into the antigen-presenting cells such as macrophage and dendritic cells (Takakura et al, 2011). Unfortunately, antigen-presenting cells will not fully digest prions. Prions escape antigen-presenting cells into the lymphatic system through the lymph fluid (Takakura et al, 2011). Then prions use the nerve endings of the peripheral nervous system to infect the central nervous system through retrograde transmission (Zabel and Avery 2015).

The role of the adaptive immune system, which specifically attacks and forms memory cells that work in cases of recurrent infections, has not been detected. No antibodies have been found for prion infectious agents (Zabel and Avery 2015). The absence of humoral immunity is due to the negative selection that eliminates B and T cells that recognize normal prion protein as self-antigen (Zabel and Avery 2015). Since the misfolded and normal forms of the prion protein share the same amino acid sequence, the misfolded form is also ignored by the adaptive immunity (Zabel and Avery 2015).

Upon prion arrival into the central nervous system, prion agents will transform PrP<sup>C</sup> into PrP<sup>SC</sup> (Sakudo et al, 2011). This transformation will be followed by PrP<sup>SC</sup> aggregation in different parts of the nervous system. Deposition of PrP<sup>SC</sup> leads to the pathological features of prion diseases, such as neuronal cell loss, vacuolation, astrocytosis, and amyloid plaques (Figure 5) (Sakudo et al, 2011). These features are followed by the appearance of symptoms and signs (White and Mallucci 2009).

### **Signs and Symptoms of Prion Diseases**

Prion diseases represent a group of conditions with strain-specific clinical signs. Clinical signs are variable in different breeds, flocks, regions, countries, prion strains, and the stages of the disease (Sakudo et al, 2011). Different strains affect different areas of the brain, for example, prions attack the cerebral cortex in CJD, cerebellum in GSS, and thalamus in FFI (Sakudo et al, 2011). The clinical phase progresses slowly over several weeks to months. It usually attacks the central nervous system, resulting in progressive neuronal degeneration and neuronal vacuolation (Prusiner 1998). Not all symptoms are always present, but usually at least one or more signs are noticeable. Prion diseases result in depression, cardiac arrhythmia, memory loss, head tremor, teeth grinding,

hyperresponsiveness, anxiety, excessive salivation, aggressiveness, pruritus, cannibalism and biting, gait or limb ataxia, and visual signs (field defects, distortion, cortical blindness) (Groschup 2013). These signs usually appear in adulthood and get worse with time, and ultimately lead to death (Groschup 2013). However, that is not always the case; sporadic CJD can appear in younger people as well.

Prions also can attack systems other than the nervous system. For instance, it has been found that the loss of prion protein is associated with insulin resistance and obesity (de Brito et al, 2017).

### **Species Barriers of Prion Diseases**

The protein-only hypothesis of prion disease explains how a mismatch of prion protein sequences for different mammals modulates the species barriers for prion transmission. The tertiary structure of PrP<sup>C</sup> is conserved among mammals (Wulf et al, 2017). Sequence similarities are 88-93% between human, bovine, and porcine prion proteins (Hammarstrom and Nystrom 2015).

The species barrier in prion diseases reflects the difficulty of the disease to be transmitted from one species to another and cause disease (Wen-Quan Zou 2013). Transmission depends on the potential for exogenous PrP<sup>SC</sup> and endogenous PrP<sup>C</sup> to interact, where interaction relies on the conformational compatibility between the two proteins (Priola 2013). Prions can be transmitted within and among species. Intraspecies transmission of prion diseases naturally occurs, but with variable degrees. For instance, sheep scrapie can be transmitted both vertically and horizontally via placental tissue, which is considered the common route for prion transmission within a sheep flock. In this manner, prions in a single sheep can transmit the infection to 30–40% of the flock

(Priola 2013). In chronic wasting disease in deer, shedding of the infectious agents in saliva, feces, and urine results in highly efficient spreading of the prion, with up to 100% of the deer becoming infected in a herd (Priola 2013). On the other hand, sporadic Creutzfeldt–Jakob disease in humans has detectable infectivity in the central nervous system (CNS) only; its transmission requires either ingestion of part of the nervous system or iatrogenic transmission (Priola 2013).

Intraspecies transmission is easier than interspecies transmission of prions. Species barriers in prion diseases are very strong, there is not a single case of naturally occurring prion disease such as sheep scrapie, CWD or sCJD, that has crossed the species barriers under normal conditions. Crossing the species barrier requires human intervention (Priola 2013). For instance, changes in the rendering process of ruminant carcasses to make meat and bone meal resulted in a successful crossing of the species barrier for prions between sheep and cattle, which is the most favored hypothesis for the origin of BSE (Priola 2013).

Species barriers to prion infection were identified by running experimental inoculations of different prion strains into different mammalian species, (Figure 6). For example, mink prion can be transmitted to hamsters but not mice, and mice are susceptible to mouse prion but highly resistant to hamster prion (Priola 2013).

Mice expressing hamster prion protein are susceptible to hamster scrapie. Transgenic mice expressing chimeric mouse/hamster prion protein showed a region of prion protein that has a major role in the transmission of hamster scrapie to mice. When amino acid residues 108 to 189 were derived from hamster, mice were susceptible to hamster prion disease (Priola 2013). However, when the sequence was derived from

mouse prion protein, mice were resistant to hamster scrapie infection (Priola 2013). Mice and hamster prion proteins are very similar, they differ in only three amino acids in the region from codon 108 to 189, thus one of these amino acids contributes to the species barrier (Figure 7) (Priola 2013).

Polymorphisms in the amino acid sequences between the species influences animal susceptibility as well as the species barrier. For example, mice expressing human prion protein are less susceptible to variant Creutzfeldt-Jakob disease compared to sporadic Creutzfeldt-Jakob disease (Hill et al, 1997). In another experiment, chronic wasting disease prions were not transmissible to mice expressing human prion protein. On the other hand, they were highly transmissible to mice expressing cervid prion protein (Kurt and Sigurdson 2016). Four single nucleotide polymorphisms in the prion protein gene were observed in pigs, one of them a single nucleotide substitution, resulted in a serine to asparagine amino acid substitution (Meng et al, 2005).

The question is how does a single amino acid difference result in a completely different secondary and tertiary structure? Certain amino acids tend to induce the misfolding more often than others (Kupfer et al, 2009). Hydrophobic interaction plays an important role in  $\beta$ -sheet formation since it brings polypeptide chains into close proximity; moreover the presence of hydrophobic amino acids in intermediate state tends to form aggregations, which may lead to initiation of disease (Kupfer et al, 2009).

Misfolding and aggregation can be initiated without the presence of infectious prion agents, which is exactly what happens in genetic and sporadic prion disease cases (Kupfer et al, 2009). Physiological conditions, such as salt concentrations and pH, are other possible factors that facilitate protein misfolding (Kupfer et al, 2009). For instance,

spider silk protein showed rapid refolding upon the reduction in sodium concentration, increase in potassium concentration and drop in the pH (Kupfer et al, 2009).

Pigs are considered resistant to prion disease since no single case of naturally occurring prion diseases has been reported yet. However, some recent *in vitro* studies did show that pigs can be infected with different prion strains. For example, scientists tried to investigate the susceptibility of swine to the CWD agent following oral or intracranial inoculation of the prion agents. Four out of ten intracranially inoculated pigs and one out of ten orally inoculated pigs were positive for prion infection (Hedman et al, 2016). *In vitro*, pigs were susceptible to both BSE and sheep-derived BSE (sheep that were experimentally infected by intracerebral inoculation with the BSE agent) (Hedman et al, 2016). Interestingly, the transmission of sheep-derived BSE was more efficient than the original cattle-BSE isolate in a transgenic mouse model expressing porcine prion protein. (Hedman et al, 2016).

The main goal of this research was to sequence the prion protein gene isolated from pig tissues and search for polymorphisms. And correlate the polymorphisms to the animal susceptibility for prion diseases. Subsequently, the sequences were compared with other prion disease susceptible and non-susceptible mammals. Because pigs live in close proximity to humans and though there are no naturally reported pig prion disease, research on pig prion protein should be given prior importance and should be continued.

## MATERIALS AND METHODS

### Sample Collection

A total of 35 feral pigs (*Sus scrofa*) tail samples were obtained from hunters in two different locations in Texas, United States. Pig tails were placed in 70% ethanol and kept at -20°C freezer to DNA extraction.

### Genomic DNA Extraction

I used the DNeasy Blood and Tissue kit from Qiagen (Hilden, Germany) to isolate whole genomic DNA from each sample. The instructions from the manufacturer were followed as described. DNA quality and quantity were evaluated using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE).

### Polymerase Chain Reaction (PCR)

Using the extracted pig genomic DNA as the template, I used PCR with previously published primers to amplify the prion gene (Martin et al, 1995). DNA was amplified using Phusion High Fidelity PCR kit obtained from New England Biolabs Inc. (Ipswich, MA). Martin et al. (1995) PCR protocol was followed for all the steps except the annealing temperature. A Fifty microliter PCR reactions were carried out in 0.5 mL tubes, each tube containing 0.5  $\mu$ L Phusion polymerase, 1  $\mu$ L of 10 mM dNTPs, 2  $\mu$ L of template DNA, 2.5  $\mu$ L each of 10  $\mu$ M PrP forward (5'CATTTGATGCTGACACCCTCTTTA3') and reverse



(5'ATGAGACACCACCACTACAGGGCT3') primers, 10 µL of 5X Phusion High Fidelity Buffer, and 31.5 µL of deionized water.

PCR cycling and running parameters were set up as the following: initial denaturation at 98 °C for 30 seconds, followed by 30 cycles of denaturation at 98 °C for 10 seconds, annealing at 60 °C for 30 seconds, and extension at 72 °C for 30 seconds. Ultimately, final elongation was at 72 °C for 10 minutes.

### **Agarose Gel Electrophoresis, PCR Purification, and Sequencing**

Gel electrophoresis was used for assessment of the PCR products. TAE buffer was used to prepare 1% agarose gels (Agarose low EEO, Thermo Fisher Scientific, Waltham, MA). Sybersafe stain was obtained from Invitrogen and used for gel staining (Carlsbad, CA). The one Kb DNA ladder, obtained from Promega, (Madison, WI), was used to verify PCR product size, and the loading dye used was from GelPilot. PCR products were submitted to Genewiz (South Plainfield, NJ), for clean-up and nucleotide sequencing.

### **Analysis**

Comparison of the nucleotide and amino acid sequences was done within wild pigs and among other mammalian species was done. Using BLAST tool of National Center for Biotechnology Information (NCBI), different amino acid sequences were obtained for the prion protein gene. These sequences included different mammalian species (Figure 11). MEGAX software (Tamura et al, 2007) and Unipro UGENE (Okonechnikov et al, 2012) were used to align different sequences, either nucleotide or amino acid. Clustal W was used, based on the length of the sequences, for the alignment. Identifying prion protein domains was done using Inter pro (Mitchell et al, 2019; Jones et

al, 2014). The three-dimensional structure of swine prion protein was obtained using SWISS-MODEL website (Waterhouse et al, 2018; Benkert et al, 2011; Bertoni et al, 2017). A neighbor-joining tree was constructed for the amino acid sequences, using MEGAX software (Tamura et al, 2007). The default settings were used to find the best model.

## **RESULTS**

### **Prion Protein Gene Detection by PCR**

I was able to isolate and amplify the prion protein gene by PCR (Figure 8). Based on the sequences obtained from Genewiz, nucleotide sequences of length 774 base pairs were obtained, and those sequences were translated into amino acid sequences of 257 residues long. However, eight samples showed either no priming or poor-quality sequences.

### **Multiple Alignment of the Amino Acid Sequence**

Multiple amino acid sequence alignment, for 27 feral pigs sequences and 25 different mammalian species sequences were aligned using MEGAX software (Tamura et al, 2007) and Unipro UGENE (Okonechnikov et al, 2012). The prion protein gene is highly conserved among mammalian species. However, polymorphisms were observed at different locations. One of the key positions is the polymorphism of the amino acids at position 224. All sequences located above the line in Figure 9 including 27 feral porcine samples, are resistant mammalian species and have lysine, except for rabbit samples that have glutamine instead. All sequences below the line in Figure 9 are susceptible and have arginine at position 224 (Figure 9).

Another interesting polymorphism of the amino acid sequence is located at position 230. Here, all susceptible animals have tyrosine, however, most resistant animals

have either alanine or tyrosine (Figure 9). However, most of the resistant animal has alanine instead of tyrosine at position 230.

One organism from those that are resistant and has tyrosine is rabbit. Interestingly, rabbits have tyrosine, but the previous residue located at position 229 is alanine, which is the only organism that has alanine instead tyrosine, of the three sequences directly above the line (Figure 9). In other words, positions 229 and 230 in resistant animals have one polar and the other non-polar amino acid, however, in resistant animals both are polar, except for fox and dogs. This means that position 224 is more reliable in determining whether an animal is resistant or susceptible, based on the amino acid sequence (Figure 9).

### **Phylogenetic Tree**

The neighbor-joining tree was obtained using MEGAX software (Saitou and Nei 1987; Kumar et al, 2018; Zuckerkandl and Pauling 1965) . The best fit substitution model is JTT +G. The branch length signifies the rate of nucleotide substitutions.

A molecular phylogenetic tree of the amino acids clusters susceptible animals such a sheep, goat, cat, and deer together and the resistant animal such as pig, horse, dog, donkey and rabbit together, except camel which seems closer to the resistant animals, as well as human, which grouped with the rabbits (Figure 10).

## DISCUSSION

The prion protein is highly conserved among mammalian species; however, some mammals are susceptible and others are resistant to infections by prion diseases (Pastore and Zagari 2007). In prion diseases, the symptoms are caused by the transition of several of the  $\alpha$ -helices in the normal form of the protein to  $\beta$ -sheets in the pathogenic form (Kupfer et al. 2009) (Figure 2). Resistance to prion diseases then may be related to the overall stability of the  $\alpha$ -helical domain of the protein. The more resistant the  $\alpha$ -helices are to transitioning to  $\beta$ -sheets, the more resistant the animal to prion disease. This is also related to the species barrier between different mammalian species. The closer the three-dimensional conformation of the prion proteins in donor and recipient animals, the easier it is to transmit prion diseases between the two (Moore et al, 2005).

There are several regions in the nucleotide sequence in the PrP<sup>C</sup> gene of all pigs that are highly conserved. Four octapeptide repeats were observed in all animals in the N terminal region. Rabbit, human, and camel sequences have a deletion mutation within the fourth repeat (Figure 11).

I was able to identify three conserved domains in the pig prion protein using Interpro (Mitchell et al, 2019; Jones et al, 2014). These were amino acid residues 1 – 30, 117 – 132, and 138 – 254. In the first and second domains there were not any interesting polymorphisms, on the other hand the third domain, residues 138 – 254, has an interesting polymorphism. Polymorphism at position 224 is conserved in the susceptible as well as the resistant animals. As mentioned earlier, all susceptible animals have

arginine and all resistant animals have lysine except rabbits, which have glutamine. This domain consists of the first  $\alpha$ -helix 144-156, the second short  $\beta$ -strand 160-164, the second  $\alpha$ -helix 174-193, and the final  $\alpha$ -helix 199-229 (Rodriguez et al, 2017).

Interestingly, amino acid residues in the 199 to 229 region also maps to the last  $\alpha$ -helical domain of prion protein (Figure 9) (Rodriguez et al, 2017).

Amino acid residues differ in their helix-forming potential, with methionine, alanine, leucine, glutamate, and lysine having a tendency to be part of  $\alpha$ -helix structures and are helix-stabilizing residues. Linear side chains with two, three, or four carbons are strongly helix-stabilizing (Lyu et al, 1991). The polymorphism observed in this work confers stability to this region, providing evidence of the effect of polymorphisms on animal susceptibility to prion (Leiro et al, 2017).

Another amino acid polymorphism is the combination of position 229 and 230, most susceptible animals have two aromatic amino acid residues. aromatic amino acid such as tyrosine has a large side chain, the presence of two tyrosine residues next to each other and subsequently two large side chains is unfavorable for  $\alpha$ -helices formation. However, resistant animals have a combination of tyrosine or another aromatic amino acid and amino acid other than aromatic residues, the combination of tyrosine and alanine is more favorable since alanine has a very small side chain compared to tyrosine, this interaction is preferable for  $\alpha$ -helices formation. Moreover, the presence of two aromatic amino acid residues result in the formation of pi-stacking that results in  $\beta$ -sheet formation.

One exception is in the canines, including dogs and foxes. The interesting observation in canines is that they have polymorphisms in positions that are highly

conserved in resistant and susceptible animals. For example, at position 219 all animals have isoleucine, except canines have valine. Also, at 181, canines have arginine instead of histidine. This change is important, although both residues are basic, histidine is neutral at physiological pH. At position 163, canines have aspartic acid instead of asparagine, at 107 they have asparagine instead of serine (Figure 12). Many factors play a role in protein structure, such as whether amino acid residues are basic, acidic, polar, or non-polar. Their interactions and locations to each other are also important. For instance, non-neighbor amino acids may interact and affect the structure of the protein. In the prion protein, amino acids 149, 208, and 212 are very close to each other and their interactions affect the overall structure. Similarly, this is also observed for residues 119, 120, 156, and 193 (Figure 13). This means these polymorphisms in canines may interact and stabilize the normal prion protein, preventing it from misfolding. Moreover, some of these polymorphisms are located in  $\alpha$ -helices, such as polymorphism 181 located in the second  $\alpha$ -helix (residues 174-193), and polymorphism 219 located within the last  $\alpha$ -helix (residues 199-229).

The molecular phylogenetic tree (Figure 10) represents the evolutionary relationships among groups of organisms. The tree is divided into two main clades, in the first clade all organisms are pigs, either from feral pigs or pig sequences from NCBI. The second clade consists of both prion-resistant and susceptible organisms. The resistant animals such as horses, dogs, and foxes are grouped together and are closer to the pigs' sequences. It is surprising that the rabbit is grouped with the susceptible organisms.

## CONCLUSION

Prion disease is a serious condition. The conversion of the cellular prion protein (PrP<sup>C</sup>) into the pathogenic isoform (PrP<sup>SC</sup>) is a crucial step (Westergard et al, 2007). This conversion is controlled by many factors such as amino acid sequence, pH, and salt concentration. My results showed that different vital amino acid polymorphisms can be used to judge the possibility for an animal to get a prion disease based on these polymorphisms.

Most mammals are susceptible, however, some are not. Pigs, horses, dogs, and rabbits are resistant animals. Amino acid polymorphisms located at position 224 maybe more diagnostic than position 230. However, combining the two positions can increase the confidence of the prediction of susceptibility.

There are more than 50 protein misfolding disease including Alzheimer, Parkinson's disease and prion diseases. The more we know about one of these diseases will help in forming a better image about other protein misfolding diseases.





Figure 1: Examination of child infected with Kuru in Papua New Guinea. Children and women were more likely to be affected with kuru, as specific kin had specific rights to certain body parts (Genetic Science Learning Center 2016 ).

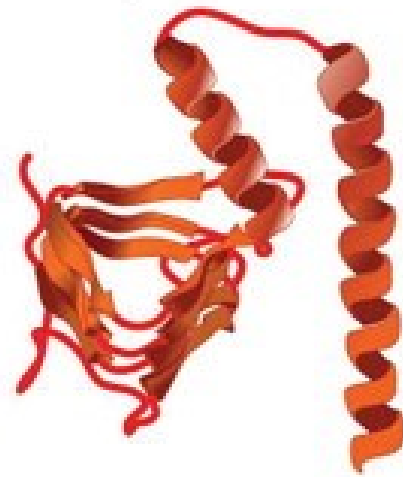
## Proposed Structure of PrP<sup>C</sup> and PrP<sup>Sc</sup>

$\alpha$ -helix 40%  
 $\beta$ -sheet 3%



**NORMAL PRION PROTEIN (PrP<sup>C</sup>)**

$\alpha$ -helix 30%  
 $\beta$ -sheet 40%



**DISEASE-CAUSING PRION (PrP<sup>Sc</sup>)**

Figure 2: Difference in  $\beta$ -sheets content in PrP<sup>C</sup> and PrP<sup>Sc</sup>. This change results in different physicochemical properties such as solubility, structure, and stability (Lee et al, 2013).

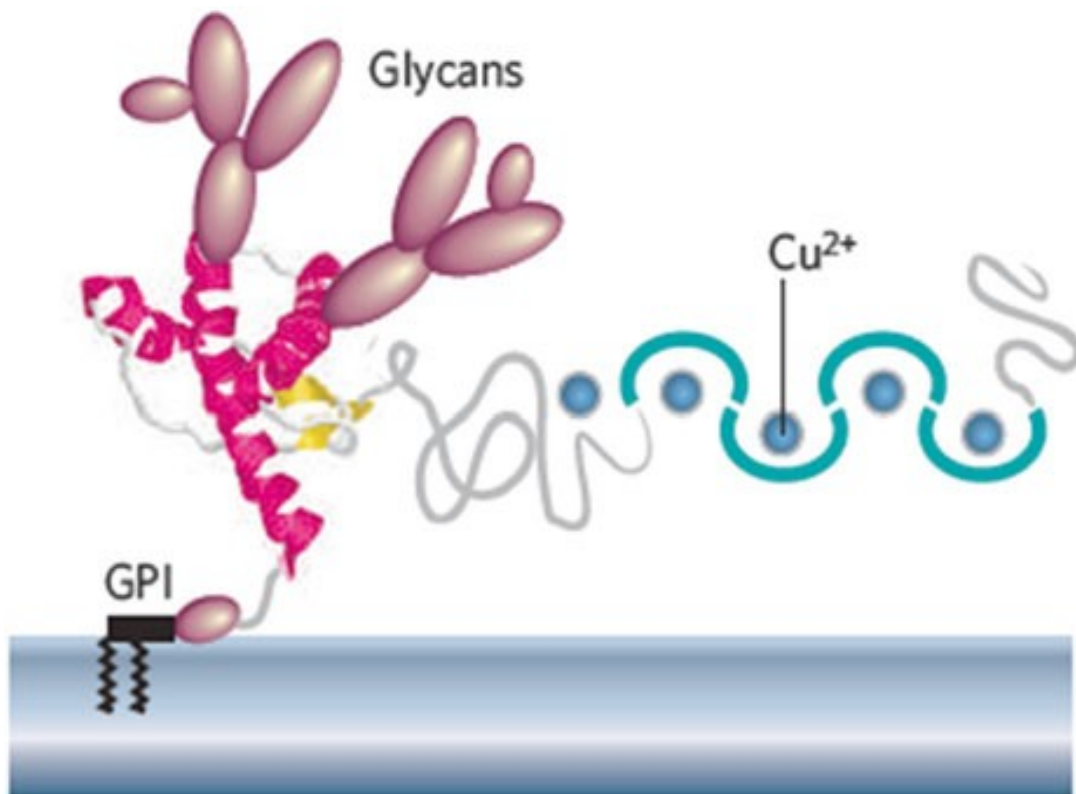


Figure 3: Mouse prion protein PrP (residues 23–231). The C-terminal structural region with a GPI anchor and non-structured N-terminal region that binds copper ions (Beckerman 2015)

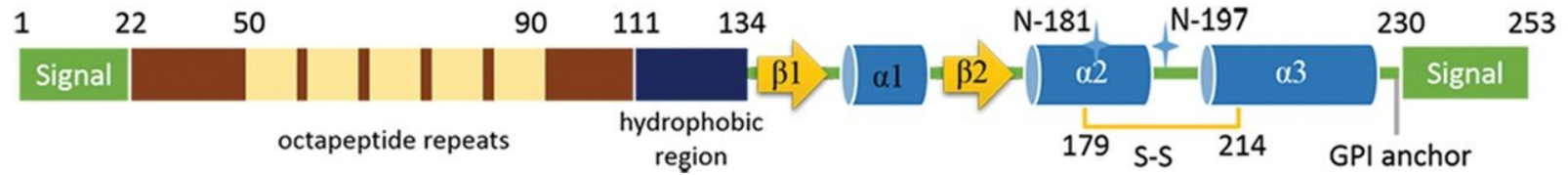


Figure 4: Human prion protein, N-terminal and C-terminal regions. The N-terminal domain of PrP is non-structured region containing five octapeptide repeats, the hydrophobic region is blue color. The C-terminal globular domain contains two  $\beta$ -sheets and three  $\alpha$ -helices. The C-terminal region contains single disulfide bridge, two N-glycosylation sites, and glycoposphatidylinositol-anchor (Zhou and Xiao 2013).

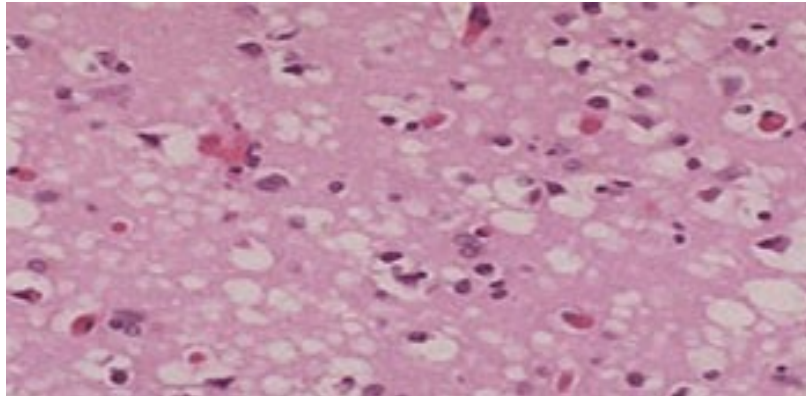


Figure 5: Histopathological lesion, vacuolation of the central nervous system in a classic CJD patient (CentersforDiseaseControl 2018).

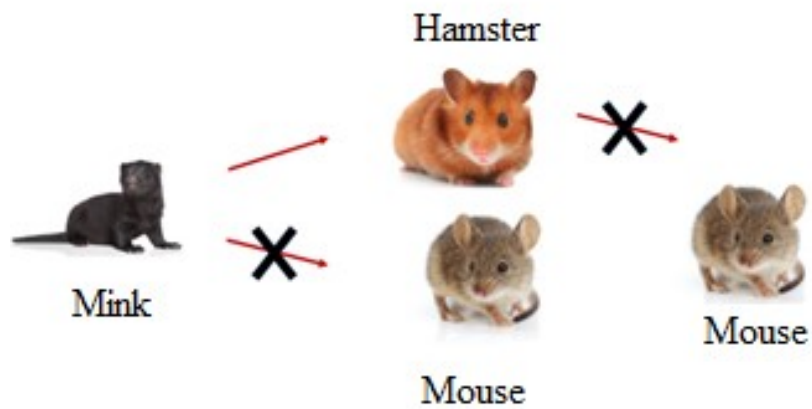


Figure 6: Species barriers to prion were identified by running experimental inoculation of different prion strains into different mammalian species. For example, mink prion can be transmitted to hamsters but not mice. And mice are highly resistant to hamster scrapie.

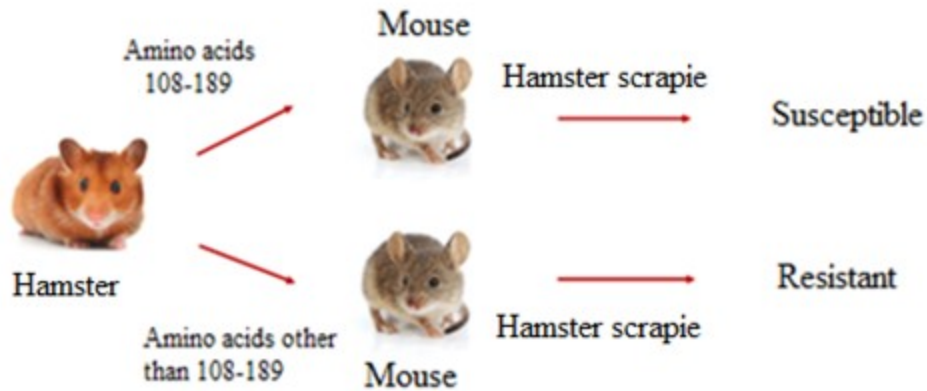


Figure 7: Transgenic mice expressing chimeric mouse/hamster prion protein showed a region of prion protein that has a major role in the transmission of hamster scrapie to mice. When amino acid residues 108 to 189 were derived from hamster, mice were susceptible to hamster prion disease. However, when the sequence was derived from mouse prion protein, mice were resistant to hamster scrapie infection (Priola 2013).

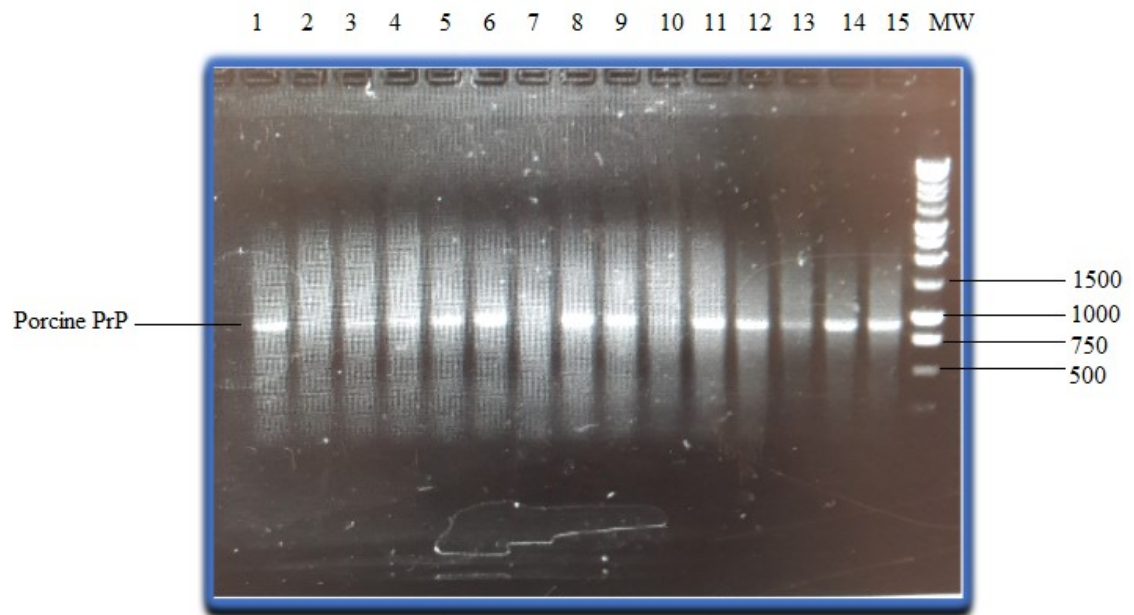


Figure 8: Agarose gel electrophoresis of PCR products of PrP<sup>C</sup> gene (774bp) for 15 feral pigs' samples.

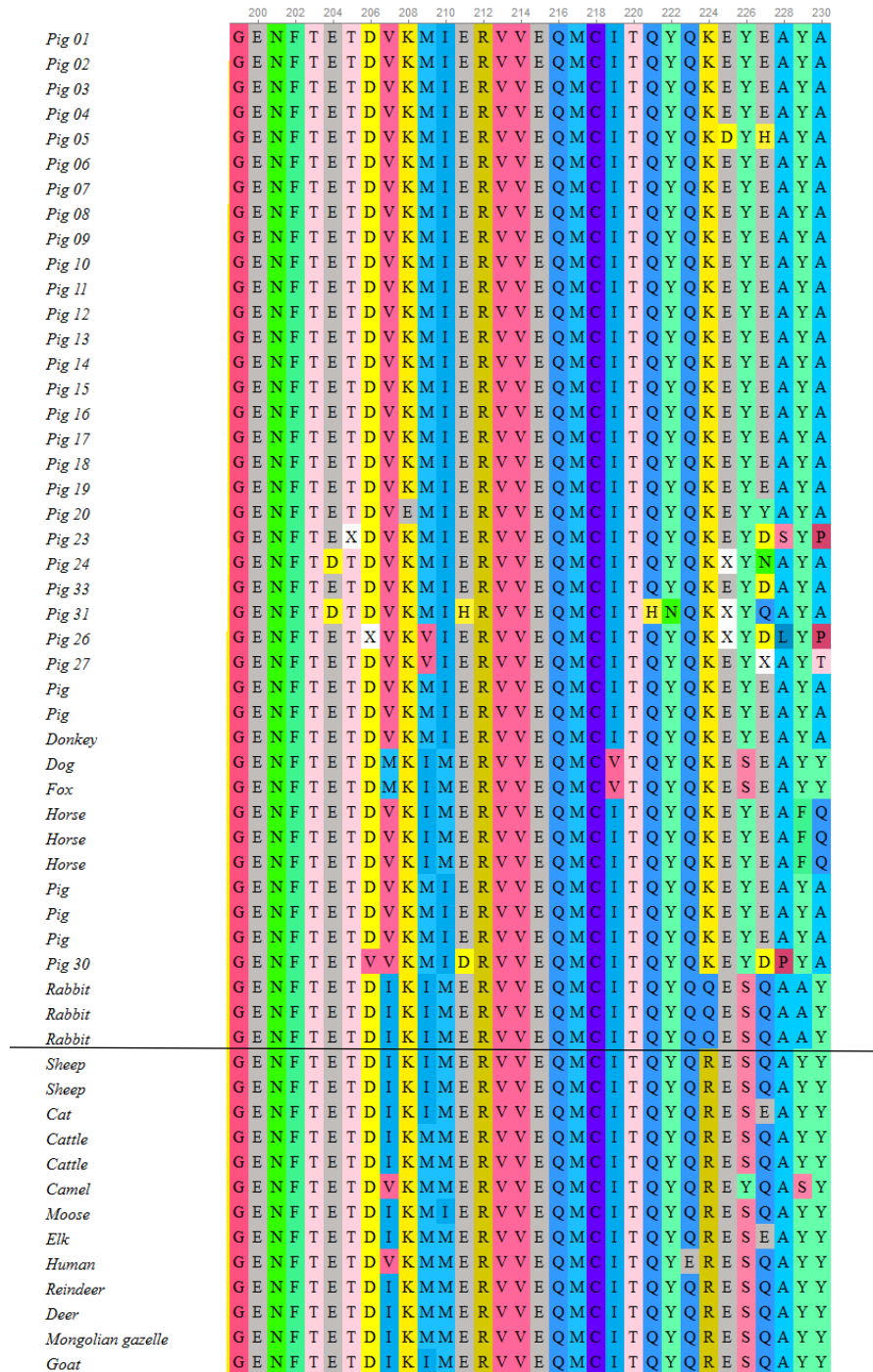


Figure 9: Multiple amino acid sequence alignment for prion protein of resistant animals, located above the line and susceptible animals are located below the line. Alignment was obtained using Unipro UGENE (Okonechnikov et al, 2012).



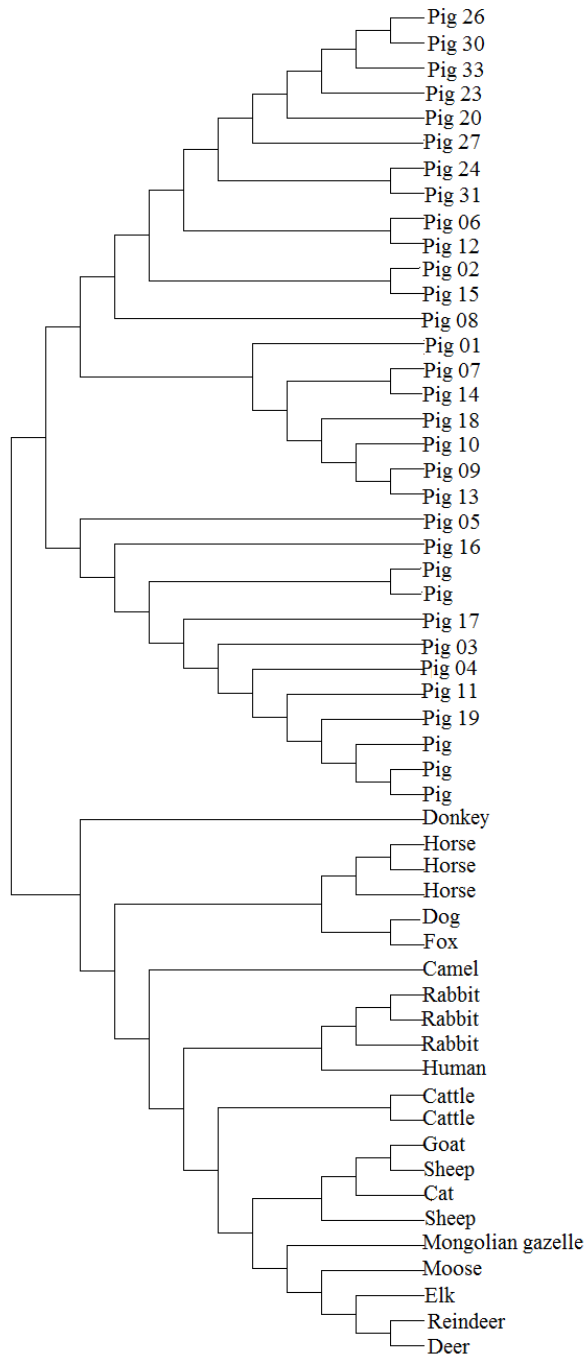


Figure 10: Neighbor-joining tree for amino acid sequences of different mammalian species including feral pig samples, produced with MEGAX software (Tamura et al, 2007).

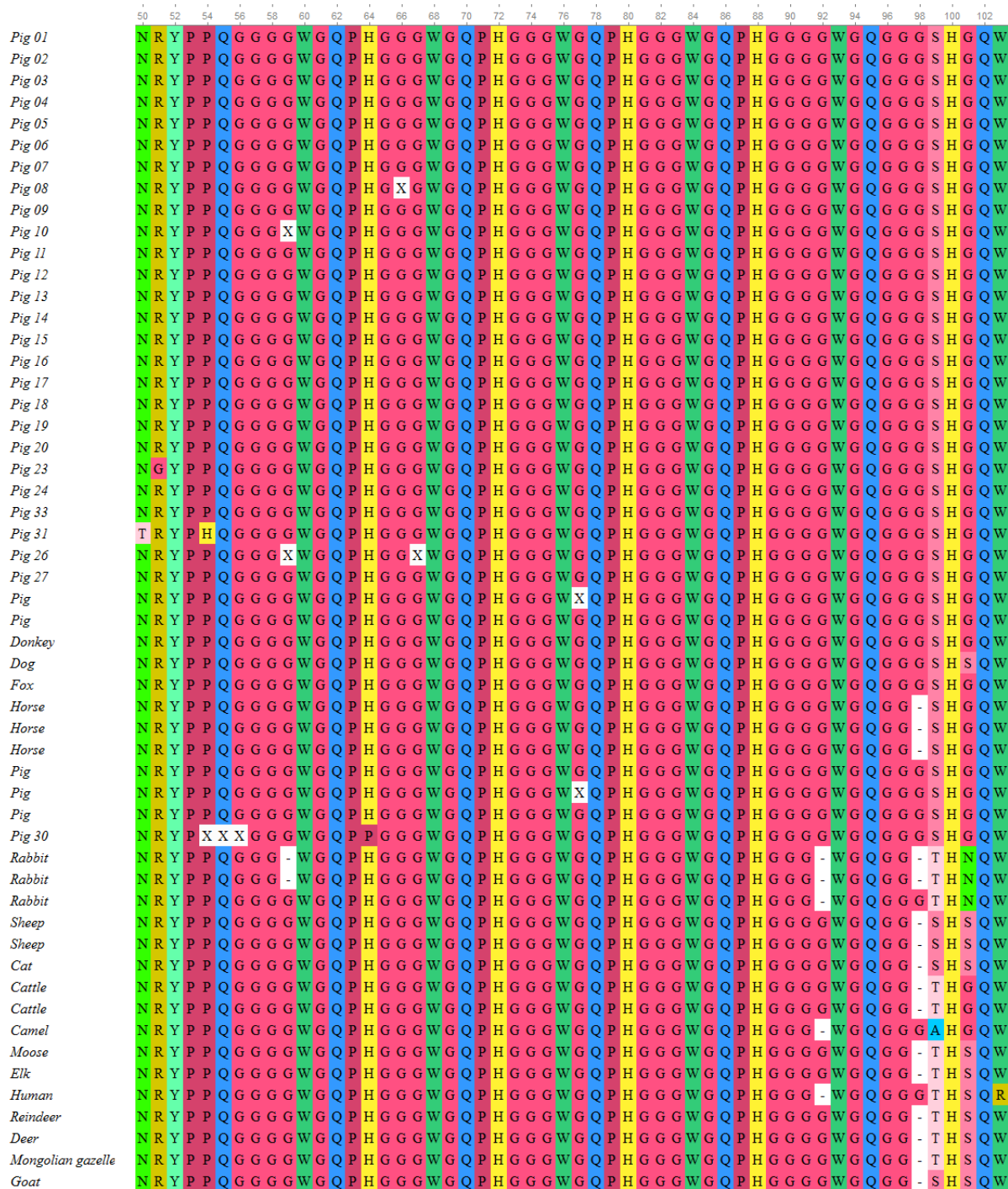


Figure 11: Four octa repeat (PHGGGWGQ) in prion protein for different species, some organisms have single amino acid deletion mutation such as human and rabbits.

Alignment was obtained using Unipro UGENE (Okonechnikov et al, 2012)

	106	107	108	162	163	178	180	181	182	216	218	219	220							
<i>Pig 01</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 02</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 03</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 04</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 05</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 06</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 07</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 08</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 09</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 10</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 11</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 12</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 13</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 14</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 15</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 16</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 17</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 18</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 19</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 20</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 23</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 24</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 33</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 31</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 26</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 27</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Donkey</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Dog</i>	P	N	K	P	Y	P	D	Q	Q	N	N	F	V	R	D	Q	M	C	V	T
<i>Fox</i>	P	N	K	P	Y	P	D	Q	Q	N	N	F	V	R	D	Q	M	C	V	T
<i>Horse</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Horse</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Horse</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Pig</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Pig</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Pig 30</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Rabbit</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Rabbit</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Rabbit</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Sheep</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Sheep</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Cat</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Cattle</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Cattle</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Camel</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Moose</i>	P	S	K	P	Y	P	N	Q	Q	N	S	F	V	H	D	Q	M	C	I	T
<i>Elk</i>	P	S	K	P	Y	P	N	Q	Q	N	T	F	V	H	D	Q	M	C	I	T
<i>Human</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Reindeer</i>	P	S	K	P	Y	P	N	Q	Q	N	T	F	V	H	D	Q	M	C	I	T
<i>Deer</i>	P	S	K	P	Y	P	N	Q	Q	N	T	F	V	H	D	Q	M	C	I	T
<i>Mongolian gazelle</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T
<i>Goat</i>	P	S	K	P	Y	P	N	Q	Q	N	N	F	V	H	D	Q	M	C	I	T

Figure 12: Prion protein polymorphisms, at position 107 all mammals have serine except canines, which have asparagine. Also, at position 163 canines have aspartic acid instead

of asparagine. At position 181 canines have arginine instead of histidine. Canines also have valine at position 219, where other mammals have isoleucine. Alignment was produced using Unipro UGENE (Okonechnikov et al, 2012).

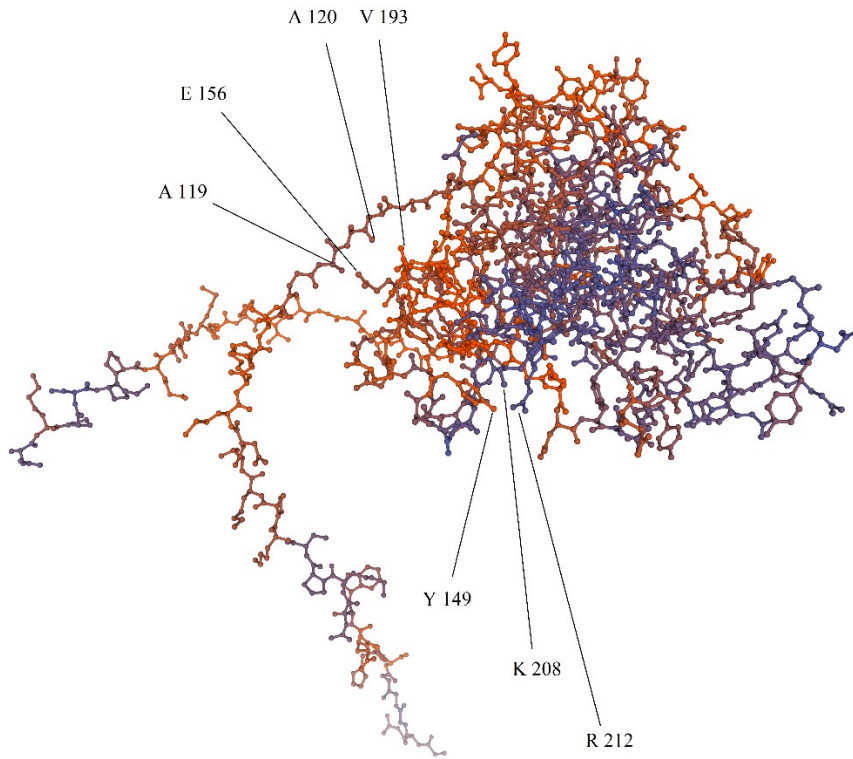


Figure 13: Three dimensional structure of pigs prion protein. This figure shows non neighbor amino acids, for example, amino acids 149, 208, and 212 are very close to each other and their interaction affects the overall structure. This is similar for amino acids 119, 120, 156, and 193. Image from SWISS-MODEL website (Waterhouse et al, 2018; Benkert et al, 2011; Bertoni et al, 2017).

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Appendix 1: Amino acid sequences of different mammalian species

Pig 01

MVKSQIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFRILG

Pig 02

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVIVLISFLLFPIVG

Pig 03

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

Pig 04

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY

RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

Pig 04

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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 06

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGGGVILFSSPPVIPLISFXLPHSG

Pig 07

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 08

MXKSHIGGWILXXFVAAWSDXGXCKKRPKPGGGXNXGGSRYPGQGSPGGNRY  
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YRENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETD  
VKMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLXXIVG

Pig 09

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQSPGGNRYP  
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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILXSSPPLILVISFPLNGFFT

Pig 10

XXKXHIXGWIXVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSXGGNRYP  
PPQGGGXWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHG  
QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY  
YRENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETD  
VKMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILVISFLLXXSVX

Pig 11

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RENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 12

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MIERVVEQMCITQYQKEYEAYAQRGXSVILFSSPPVIFPISFFFLIVG

Pig 13

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
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WVKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYR  
RENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 14

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RENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 15

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
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WVKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYR  
RENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVIVLISFLLFVIVG



Pig 16

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Pig 17

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Pig 18

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Pig 19

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Pig 20

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RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
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Pig 23

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Pig 24

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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTDGENFTD TDV  
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Pig 33

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WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY  
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Pig 31

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QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY  
YRENMYRYPNQVYYRVPVDHYSNQNSFVHDCVNITVNQHTVTTTTTKGENFTDTD  
VKMIHRVVEQMCITHNQKXYQAYALRRASVILFSSLPVILLISFLGFFIPG

Pig 26

MVKXHIGGWIXVLFVAAWSDIGLGEKRPKLGGGWWTNGGSRYPWQGSPGGNRY  
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QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYDDR  
YYRENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTET  
XVKVIERVVEQMCITQYQKXYDLYPPIGGVVIPLYAPPVIPGISCFSLVGG

Pig 27

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QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY  
YRENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETD  
VKVIERVVEQMCITQYQKEYXAYTQIGASVILFSSPPVIFLLCYVLFFIPG

Pig 30

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QWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYDDR  
YYRENMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTET  
VVKMIDRVVEQMCITQYQKEYDPYAGGWAIIVILFHPPSVRLFISFSFLIPEG

*Oryctolagus cuniculus* (Gen Bank)- European rabbit

MAHLGYWMLLLFVATWSDVGLCKKRPKPGGGWNTGGSRYPGQSSPGGNRYPP  
QGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGTHNQWGKP  
SKPKTSMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYRENM  
YRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDIKIMER  
VVEQMCITQYQQESQAAYQRAAGVLLFSSPPVILLISFLIFLIVG

*Oryctolagus cuniculus* (Gen Bank)- European rabbit

LLLFVATWSDVGLCKKRPKPGGGWNTGGSRYPGQSSPGGNRYPPQGGGWGQP  
HGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGTHNQWGKPSKPKTSMKH  
VAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYRENMYRYPNQVY  
YRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDIKIMERVVEQMCITQ  
YQQESQAAYQRAAGVLLFSSPPVILLISFLIFLIVG

*Oryctolagus cuniculus* (Gen Bank)- European rabbit

MAHLGYWMLLLFVATWSDVGLCKKRPKPGGGWNTGGSRYPGQSSPGGNRYPP  
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KPSKPKTSMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYRE  
NMYRYPNQVYYRPPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDIKIM  
ERVVEQMCITQYQQESQAAYQRAAGVLLFSSPPVILLISFLIFLIVG

*Sus scrofa* (Gen Bank)- Pig

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Sus scrofa* (Gen Bank)- Pig

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWXQPHGGGWGQPHGGGGWGQGGGSHGQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Sus scrofa* (Gen Bank)- Pig

MVKNHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
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WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRPVDQYSNQNSFVHDCVNITVKQHTVTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Ovis aries* (Gen Bank)- Sheep

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHSQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY

RENMYRYPNQVYYRVPDQYSNQNNFVHDCVNITVKLHTVTTTTTKGENFTETDIK  
IMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Felis catus* (Gen Bank)- Domestic cat

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHSQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRVPDQYSNQNNFVHDCVNITVKQHTVTTTTTKGENFTETD  
IKIMERVVEQMCITQYQRESEAYYQRGASVILFSSPPVILLISFLIFLIVG

*Bos Taurus* (Gen Bank)- Cattle

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHGQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY  
RENMHRYPNQVYYRVPDQYSNQNNFVHDCVNITVKEHTVTTTTTKGENFTETDIK  
MMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Bos Taurus* (Gen Bank)- Cattle

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGW  
GQGGTHGQWNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHF  
GSDYEDRYRENMHRYPNQVYYRVPDQYSNQNNFVHDCVNITVKEHTVTTTTTK  
GENFTETDIKMMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLI  
VG

*Alces alces shirasi* (Gen Bank)- Moose

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRPPVDQYNNQNTFVHDCVNITVKQHTVTTTTTKGENFTETD  
IKMIERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Cervus elaphus nelsoni* (Gen Bank)- Rocky Mountain elk

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRPPVDQYNNQNTFVHDCVNITVKQHTVTTTTTKGENFTETD  
IKMMERVVEQMCITQYQRESEAYYQRGASVILFSSPPVILLISFLIFLIVG

*Rangifer tarandus granti* (Gen Bank)- Reindeer

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLSGYMLGSAMSRPLIHFGNDYEDRYY  
RENMYRYPNQVYYRPPVDQYNNQNTFVHDCVNITVKQHTVTTTTTKGENFTETDI  
KMMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Hydropotes inermis argyropus* (Gen Bank)- Water deer

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRPPVDQYNNQNTFVHDCVNITAKQHTVTTTTTKGENFTETD  
IKMMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Procapra gutturosa* (Gen Bank)- Mongolian gazelle

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGTHSQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRPVDQYSNQNNFVHDCVNITVKQHTVTTTTKGENFTETD  
IKMMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Capra hircus* (Gen Bank)- Goat

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHSQ  
WnkpskpktnmkhvAGAAAAGAVVGSLLGGYMLGSAMSRPLIHFGNDYEDRY  
RENMYRYPNQVYYRPVDQYSNQNNFVHDCVNITVKQHTVTTTTKGENFTETDI  
KIMERVVEQMCITQYQRESQAYYQRGASVILFSPPPVILLISFLIFLIVG

*Ovis aries* (Gen Bank)- Sheep

MVKSHIGSWILVLFVAMWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PPQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHSQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPNQVYYRPVDQYSNQNNFVHDCVNITVKQHTVTTTTKGENFTETD  
IKIMERVVEQMCITQYQRESQAYYQRGASVILFSSPPVILLISFLIFLIVG

*Sus scrofa* (Gen Bank)- Pig

MVKSHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRY  
PQGGGGWGQPHGGGWGQPHGGGWXQPHGGGWGQPHGGGGWGQGGGSHGQ  
WnkpskpktnmkhvAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRY



RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Sus scrofa* (Gen Bank)- Pig

MVKNHIGGWILVLFVAAWSDIGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Equus asinus* (Gen Bank)- Donkey

MVKSHVGGWILVLFVATWSDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQ  
WNKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYY  
RENMYRYPNQVYYRVPDQYSNQNSFVHDRVNITVKQHTVTTTTTKGENFTETDV  
KMIERVVEQMCITQYQKEYEAYAQRGASVILFSSPPVILLISFLLFLIVG

*Camelus dromedaries* (Gen Bank)- Camel

ILVLFVVTWSDVGLCKKRPKPGGGWNTGGSRYPGQGSPGGNRYPQGGGGWG  
QPHGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQGGGAHGQWNKPSKPKTS  
MKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYERYRENMYRYPNQ  
VYYKVPDQYSNQNSFVHDCVNITVKQHTVTTTTTKGENFTETDVKMMERVVEQ  
MCITQYQREYQASYGRGASVIFSSPPVILLISFSS

*Canis lupus familiaris* (Gen Bank)- Dog

MVKSHIGGWILLVLFVATWSDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHSQ

WGKPNKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPDQVYYRVPDQYSNQNNFVRDCVNITVKQHTVTTTTTKGENFTETD  
MKIMERVVEQMCVTQYQKESEAYYQRGASAILFSPPPVILLISLLILLIVG

*Vulpes vulpes* (Gen Bank)- Red fox

MVKSHIGGWILLLFVATWSDVGLCKKRPKPGGWNTGGGSRYPGQSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGGSHGQ  
WGKPNKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRY  
YRENMYRYPDQVYYRVPDQYSNQNNFVRDCVNITVKQHTVTTTTTKGENFTETD  
MKIMERVVEQMCVTQYQKESEAYYQRGASAILFSP

*Equus caballus* (Gen Bank)- Horse

MVKSHVGGWILVLFVATWSDVGLCKKRPKPGGWNTGGGSRYPGQSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHGQW  
NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYR  
ENMYRYPNQVYYRVPVSEYSNQNNFVHDCVNITVKQHTVTTTTTKGENFTETDVKI  
MERVVEQMCITQYQKEYEAFQQRGASVVLFSPPVLLIS

*Equus caballus* (Gen Bank) - Horse

MVKSHVGGWILVLFVATWSDVGLCKKRPKPGGWNTGGGSRYPGQSPGGNRYP  
PQGGGGWGQPHGGGWGQPHGGGWGQPHGGGWGQPHGGGGWGQGGSHGQW  
NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYR  
ENMYRYPNQVYYRVPVSEYSNQNNFVHDCVNITVKQHTVTTTTTKGENFTETDVKI  
MERVVEQMCITQYQKEYEAFQQRGASVVLFSPPVLLISFLIFLIVG

*Equus caballus* (Gen Bank) - Horse

MVKSHVGGWILVLFVATWSDVGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGGWGQPHGGGGWGQPHGGGGWGQPHGGGGWGQGGSHGQW  
NKPSKPKTNMKHVAGAAAAGAVVGGLGGYMLGSAMSRPLIHFGNDYEDRYR  
ENMYRYPNQVYYRVPVSEYSNQNNFVHDCVNITVKQHTVTTTTKGENFTETDVKI  
MERVVEQMCITQYQKEYEAFQQRGASVVLFSPPVLLIFFLIFLIVG

*Homo sapiens* (Gen Bank)- Human

MANLGCWMLVLFVATWSDLGLCKKRPKPGGWNTGGSRYPGQGSPGGNRYP  
PQGGGGWGQPHGGGGWGQPHGGGGWGQPHGGGGWGQPHGGGGWGQGGGTHSQRNK  
PSKPKTNMKHMAGAAAAGAVVGGLGGYVLSAMSRPIIHFGSDYEDRYREN  
MHRYPNQVYYRPMDEYSNQNNFVHDCVNITIKQHTVTTTTKGENFTETDVKMM  
ERVVEQ

Appendix 2: An Institutional Animal Care and Use Committee (IACUC)



FORT HAYS STATE  
UNIVERSITY

*Forward thinking. World ready.*

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Institutional Animal Care and Use Committee

To: Eric Gillock

From Yasuhiro Kobayashi, Institutional Animal Use and Care Committee Chair



Re: IACUC protocol (17-0004EX) titled: *Polymorphisms in the PrP prion protein gene in domestic pigs from the FHSU farm*

November 29, 2016

The Institutional Animal Care and Use Committee has reviewed your IACUC protocol application titled: *Polymorphisms in the PrP prion protein gene in domestic pigs from the FHSU farm*, and determined it to be in compliance with all USDA and PHS regulations and requirements and approved.

This approval is for the number and species of animals you listed in the protocol. Your approval will be in effect until November 28, 2019.

Please note that the IACUC is required to review and approve, prior to initiation, proposed modifications to an approved protocol.

All approved research protocols must be updated annually, and must be reviewed by the IACUC every three years. All teaching activities using vertebrate animals are reviewed annually.

IACUC approved activities may be subject to further review and approval by university officials; however, those officials may not approve an activity involving the care and use of animals if it has not been approved by the IACUC.

The Principal Investigator is responsible for following federal guidelines and university policies and procedures regarding the care and use of animals.

Please feel free to contact me if there are any questions or concerns regarding the committee's decision on your IACUC protocol.

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Thesis: p<sup>sc</sup> prion protein gene polymorphisms in the alpha-helical region in local pigs from Texas

Author: Maram Alsmady

Signature: 

Date: 05/16/2019