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Molecular Cloning and Sequence Analysis of Prion Protein Gene of Dezhou Donkey in China

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Abstract: Transmissible Spongiform Encephalopathy (TSE) or prion disease has been reported in many herbivores but not in *equus* and the species barrier might be playing a role in resistance of these species to the disease. Therefore, analysis of genotype of prion protein (PrP) in these species may help understand the transmission of the disease. Dezhou donkey is a species of *equus* reared widely in China for service, food and medicine but its PrP gene has not been studied. Based on the reported PrP sequence in GenBank we designed primers and amplified, cloned and sequenced the PrP gene of Dezhou donkey. The sequence analysis showed that the Dezhou donkey PrP gene was consisted of an open reading frame of 767 nucleotides encoding 256 amino acids. Sequence comparison showed that the PrP gene of Dezhou donkey and horse shared 91.53% homology in nucleic acids and 94.03% homology in amino acids. Amino acid residues unique to donkey as compared with horse, mink, bovine, goat, human, elk and hamster were identified. The results provided the PrP gene data for an additional *equus* species, which should be useful to the study of the species barrier of TSE.

Key words: Dezhou donkey, prion gene, molecular cloning, sequence analysis, TSE

Introduction

Transmissible Spongiform Encephalopathy (TSE) is a group of sub-acute infectious diseases in human and animals including scrapie in ovine, bovine spongiform encephalopathy in cattle, chronic wasted disease in deer, transmissible mink encephalopathy in mink, feline spongiform encephalopathy in cat and Creutzfeldt-Jakob disease in human (Prusiner, 1991; Prusiner , 1997). It is understood that the key of development of these diseases is the transformation of a cellular prion protein (PrP^{c}) into PrP^{sc}, a diseased form of PrP characterized by a β-sheet-rich topology and anti-proteolytic property. Misform PrP^{sc} has a tendency of aggregation, shaping first into short amyloidal fiber and then aggregated amyloidal spot before becoming the spongiform-like degenerative changes in brain, which can be identified by clinic detection.

Dezhou donkey is a domestic breed of donkey widely reared in northern China. It is a species of *asinus*, belonging to *Equus*, Equide of Perissodactyla, by biological classification. Dezhou donkey is mainly used for service and meat. Since the outbreak of the BSE, beef has increasingly been substituted

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by meat from other animals including donkey. In addition, donkey skin is used as the main ingredient of Ajiao, an essential blood-nourishing traditional Chinese medicine that has been certified by FDA in the USA and is widely exported to European and American market. Donkey is herbivore, like bovine and ovine. While both bovine and ovine are susceptible to the prion disease, up to the present there has been no report of the TSE cases in donkey as well as horse. It was thought that the species barrier might be playing a role in resistance of these species to the prion disease.

Prion species barrier is a phenomenon of one species being able to protect the prion infection from other species (Hill and Collinge, 2002). There are species particularity and strain particularity of prion in the process of prp^{sc} propagation. The species particularity is that one species can protect the prion attack from the other species, representing the prolonged preclinical period or even no occurrence of the disease. The essential reason of species particularity is the difference in primary and secondary structure of PrP between the recipient and host species(Hill *et al.*, 2000). Until now, the PrP gene has been cloned in human and over 30 other animals (Wopfner *et al.*, 1997). The DNA of PrP is consisted of a single exon and its coded protein is highly conserved, with human and many species of animals sharing 92.9-99.6% of homology in PrP amino acids. Variation or the polymorphism of PrP gene in human being, mouse, sheep, bovine and deer is considered to be associated with the prion disease (Liberski and Brown, 2004; Aguzzi and Polymenidou, 2004).

Studies on the PrP gene in *equus* are very limited. The available data showed that the PrPs of the domestic horse (*Equus familiaris caballus*) and the falabella horse (*Equus przewalskii caballus*) are identical on the amino acid level and have two substitutions compared to the zebra PrP (*Equus quagga boehmi*) (Wopfner *et al.*, 1997). However, there is no report about the PrP gene in donkey. In the present study, the authors cloned and sequenced the PrP gene of Dezhou donkey and analyzed the sequence of the PrP gene with that from other animals. The results provided the PrP gene data for an additional *equus* species, which should be useful to the study of the species barrier of TSE and also of interest to public healthy.

Materials and Methods

Biological Materials

Genomic DNA was extracted from peripheral blood of six Dezhou donkeys using a Genomic DNA Rapid Isolation Kit for Blood (Biodev, USA) according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

Each DNA sample was PCR-amplified for the PrP gene in a final reaction volume of 50 μL containing 250 ng of genomic DNA, 20 pmol of each primer, 250 uM of dNTP (Pharmacia) and 1.5 unit of Taq polymerase (Perkin Elmer Cetus). The primers were deduced from *white-tail deer* PrP sequence in GenBank (accession number AY360089), which were 5'-ATGGTGAAAAGCCACATAGGC-3' for sense primer and 5'-GAAGATAATGAAAACAGGAAG-3' for anti-sense primer. The PCR procedure comprised of an initial denaturation at 94°C for 5 min and 30 cycles of denaturation at 94°C for 45 sec, annealing at 58 °C for 50 sec and elongation at 72°C for 50 sec. Finally, an extension step at 72°C for 10 min was performed (Yang *et al.*, 2005; Zhang *et al.*, 2004; Wang *et al.*, 1998).

Cloning, sequencing and analysis of the amplified PrP products

Following confirmation of the amplified DNA fragment of 767 bp on a 1% agarose gel with DNA marker (DL2000, TAKALA), the PCR products were cloned into T-easy vectors (Promega, USA) and the propagated plasmids were prepared using Qiagen Plasmids Mini Kit (Qiagen, UK). Four positive clones were identified from each sample by restriction enzyme digestion with *Eco*RI and sequenced in both directions on an ABI Prism 377 automated DNA sequencer (Applied Biosystems, Foster City, USA). The nucleotide and amino acid sequences were analyzed using the DNAMAN software.

Results and Discussion

The amplified PCR product of Dezhou donkey PrP gene was 767 bp in size, similar to PrP gene reported in other animals (Fig. 1).

Sequencing of the amplified PCR fragments revealed a single open reading frame of 767 nucleotides encoding 256 amino acids. The newly identified sequence has been deposited into GenBank under the accession number AY968590.

Sequence comparison showed that the Dezhou donkey PrP gene had 91.53% homology in nucleic acid sequence and 94.03% homology in amino acids with that of domestic horse (GenBank AF117317) (Fig. 2). Dezhou donkey PrP gene also shared high homology in both nucleic acid and amino acid sequences with those from other mammals, including elk, human, mink, bovine, goat and hamster (Table 1).

In keeping with the structure of PrP in other animals, Dezhou donkey PrP had an N-terminal 23 amino acid signal peptide. This was followed by a glycine and proline-rich region (24-96)

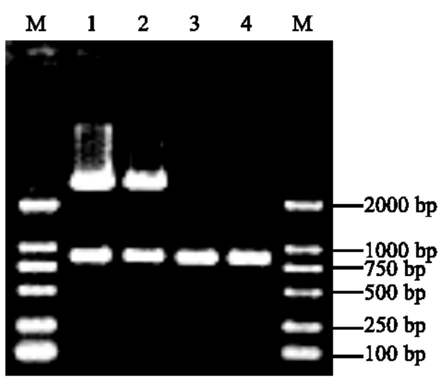

Fig. 1: Agarose gel electrophoresis of recombinant T-easy-vectors following digestion with *Eco*RI (M: marker DL2000; 1 and 2: *Eco*RI digested recombinant T-easy-vectors; 3 and 4: PCR products using recombinant T-easy-vectors as template)

Table 1: Homologies of PrP between Dezhou donkey and other species

Species		Horse	Elk	Human	Mink	Bovine	Goat	Hamster
Dezhou donkey	Nucleotide (% identity)	91.53	89.90	88.99	88.24	87.56	86.92	83.07
	Amino acid (% identity)	94.03	93.39	91.05	90.66	90.66	87.16	85.99

```
Dezhou don   .......... .......... .......... .......... ..........
Horse        .......... .......... .......... .......... ..........
Mink         .......... .......... .......... .......... ..........
Bovine       .......... .......... .......... .......... ..........
Goat         .......... .......... .......... .......... ..........
Human        .......... .......... .......... .......... ..........
Elk          .......... .......... .......... .......... ..........
Hamster      CTGATGCCAT GACTGTCACC CATTTGTTCT GCAAAAAATG TCAAGTGGCT

Dezhou don   .......... .......... .......... ..........
Horse        .......... .......... .......... .......... ..........
Mink         .......... .......... .......... .......... ..........
Bovine       .......... .......... .......... ........TG CTGACACCCT
Goat         .......... .......... .......... ...TATGATG CTGACACCCT
Human        .......... .......... .......... .......... ..........
Elk          .......... .......... .......... .......... ...ACACCCT
Hamster      TCAGCCTGCG TGCTGGACAG TGTTGTGTTG TTGGAGTATA CTGACGCCTT

Dezhou don   .......... .......... ...ATGGTGA AAAGCCACGT AGGCGGCTGG
Horse        .......... .......... .......... .......... ..........
Mink         .......... .......... ...ATGGTGA AAAGCCACAT AGGCAGCTGG
Bovine       CTTTATTTTG CAGATAAGTC ATCATGGTGA AAAGCCACAT AGGCAGTTGG
Goat         CTTTATTTTG CAGAGAAGTC ATCATGGTGA AAAGCCACAT AGGCAGTTGG
Human        .......... .......... ...ATGGTGA AAAGCCACAT AGGCAGCTGG
Elk          CTTTATTTTG CAGATAAGTC ATCATGGTGA AAAGCCACAT AGGCAGCTGG
Hamster      GTTCTTCATT TTGCAGATTA GCCATCATGG CAA...ACCT TAGCTACTGG

Dezhou don   ATTCTGGTTC TCTTTGTGGC CACATGGAGT GACGTGGGGC TCTGCAAGAA
Horse        .......... .........A CATGGAGT GACGTGGGGC TCTGCAAGAA
Mink         CTCCTGGTTC TCTTTGTGGC CACATGGAGT GACATTGGCT TCTGCAAGAA
Bovine       ATCCTGGTTC TCTTTGTGGC CATGTGGAGT GACGTGGGCC TCTGCAAGAA
Goat         ATCCTGGTTC TCTTTGTGGC CATGTGGAGT GACGTGGGCC TCTGCAAGAA
Human        ATCCTAGTTC TCTTTGTGGC CATGTGGAGT GACGTTGGCC TCTGCAAGAA
Elk          ATCCTAGTTC TCTTTGTGGC CATGTGGAGT GACGTCGGCC TCTGCAAGAA
Hamster      CTGCTGGCAC TCTTTGTGGC TACGTGGACT GATGTTGGCC TCTGCAAGAA

Dezhou don   GCGACCGAAG CCT......G GAGGATGGAA CACTGGGGGG AGCCGATACC
Horse        GCGACCGAAG CCT...GGAG GAGGATGGAA CACTGGGGGG AGCCGATACC
Mink         GCGGCCCAAG CCT...GGAG GAGGCTGGAA CACTGGGGGG AGCCGATACC
Bovine       GCGACCAAAA CCTGGA.G.G AGGATGGAAC ACTGGGGGGA GCCGATACCC
Goat         GCGACCAAAA CCTGGC.G.G AGGATGGAAC ACTGGGGGGA GCCGATACCC
Human        GCGACCAAAA CCTGGA.G.G AGGATGGAAC ACTGGGGGGA GCCGATACCC
Elk          GCGACCAAAA CCTGGA.G.G AGGATGGAAC ACTGGGGGGA GCCGATACCC
Hamster      GCGGCCAAAG CCT....G.G AGGGTGGAAC ACTGGTGGAA GCCGATACCC
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Dezhou don   CAGGGCAGGG TAGTCCTGGA GGCAACCGCT ATCCACCCCA GGGAGGGGGC
Horse        CCGGGCAGGG CAGTCCTGGA GGCAACCGCT ACCCACCCCA GGGCGGTGGC
Mink         CAGGGCAGGG CAGTCCTGGA GGCAACCGCT ACCCACCCCA GGGTGGTGGC
Bovine       AGGACAGGGC AGTCCTGGAG GCAACCGTTA TCCACCTCAG GGAGGGGGTG
Goat         GGGACAGGGC AGTCCTGGAG GCAACCGCTA TCCACCTCAG GGAGGGGGTG
Human        GGGACAGGGA AGTCCTGGAG GCAACCGCTA TCCACCTCAG GGAGGGGGTG
Elk          GGGACAGGGA AGTCCTGGAG GCAACCGCTA TCCACCTCAG GGAGGGGGTG
Hamster      TGGGCAGGGC AGCCCTGGAG GCAACCGTTA CCCACCTCAG GGTGGTGGCA

Dezhou don   GGCTGGGGAC AGCC.C.C.A .C.G.G.A.G .G.T.G.GCT GGGGACAGCC
Horse        GGCTGGGGTC AACC.C.C.A .T.G.G.T.G .G.T.G.GTT GGGGTCAGCC
Mink         GGCTGGGGCC AGCC.C.C.A .C.G.G.G.G .G.T.G.GCT GGGGACAGCC
Bovine       GCTGGGGTCA GCCCCATGGA GGTGGCTGGG GCCAGCCTCA TGGAGGTGGC
Goat         GCTGGGGTCA GCCC...... .......... ........CA TGGAGGTGGC
Human        GCTGGGGTCA GCCC...... .......... ........CA TGGAGGTGGC
Elk          GCTGGGGTCA GCCC...... .......... ........CA TGGAGGTGGC
Hamster      CCTGGGGGCA ACCC.C.A.T .G.G.T.G.G .T.G.G.ATG GGGACAGCCC

Dezhou don   CCACGGAGGC GGCTGGGGAC AGCCCCACGG TGGCGGCTGG GGACAGCCCC
Horse        CCATGGTGGT GGCTGGGGTC AGCCGCATGG TGGTGGTTGG GGACAGCCCC
Mink         CCACGGGGGT GGCTGGGGAC AGCCCCACGG GGGTGGCTGG GGACAGCCGC
Bovine       TGGGGCCAGC CTCATGGAGG TGGCTGGGGT CAGCCCCATG GTGGTGGCTG
Goat         TGGGGCCAAC CTCATGGAGG TGGCTGGGGT CAGCCCCATG GTGGTGGCTG
Human        TGGGGCCAAC CTCATGGAGG TGGCTGGGGT CAGCCCCATG GTGGTGGCTG
Elk          TGGGGCCAAC CTCATGGAGG TGGCTGGGGT CAGCCCCATG GTGGTGGCTG
Hamster      CATGGTGGTG GCTGGGGACA ACCTCATGGT GGTGGTTGGG GTCAGCCCCA

Dezhou don   ATGGTGGCGG AGGCTGGGGT CAAGGTGGTG GCT....... .CCCACGGTC
Horse        ATGGTGGTGG AGGCTGGGGT CAA...GGTG GCT....... .CCCATGGTC
Mink         ATGGTGGCGG TGGCTGGGGT CAAGGTGGTG GGA....... .GCCACGGTC
Bovine       GGGACAGCCA CATGGTGGTG GAGGCTGGGG TCAAGGTGGT ACCCACGGTC
Goat         GGGACAGCCA CATGGTGGTG GAGGCTGGGG TCAAGGTGGT AGCCACAGTC
Human        GGGACAGCCA CATGGTGGTG GAGGCTGGGG TCAAGGTGGT ACCCACAGTC
Elk          GGGACAGCCA CATGGTGGTG GAGGCTGGGG TCAAGGTGGT ACCCACAGTC
Hamster      TGGTGGTGGC TGGGGTCAAG GAGGTGGCAC CCACAATCAG TGGAACAAGC

Dezhou don   AGTGGAACAA GCCCAGTAAG CCGAAAACCA ACATGAAGCA TGTGGCAGGC
Horse        AGTGGAACAA GCCCAGTAAG CCAAAAACCA ACATGAAGCA TGTGGCAGGA
Mink         AGTGGGGCAA GCCCAGTAAG CCTAAAACCA ACATGAAGCA TGTGGCGGGT
Bovine       AATGGAACAA ACCCAGTAAG CCAAAAACCA ACATGAAGCA TGTGGCAGGA
Goat         AGTGGAACAA GCCCAGTAAG CCAAAAACCA ACATGAAGCA TGTGGCAGGA
Human        AGTGGAACAA GCCCAGTAAA CCAAAAACCA ACATGAAGCA TGTGGCAGGA
Elk          AGTGGAACAA GCCCAGTAAA CCAAAAACCA ACATGAAGCA TGTGGCAGGA
Hamster      CCAGTAAGCC AAAAACCAAC ATGAAGCATG TGGCAGGTGC GGCTGCGGCT
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Dezhou don	GCCGCTGCAG	CTGGAGCAGT	GGTAGGGGGC	CTCGGCGGTT	ACATGCTGGG
Horse	GCTGCGGCAG	CTGGAGCAGT	GGTTGGGGGC	CTCGGCGGCT	ACATGCTGGG
Mink	GCCGCAGCAG	CCGGGGCGGT	TGTGGGGGGC	CTGGGCGGCT	ACATGCTGGG
Bovine	GCTGCTGCAG	CTGGAGCAGT	GGTAGGGGGC	CTTGGTGGCT	ACATGCTGGG
Goat	GCTGCTGCAG	CTGGAGCAGT	GGTAGGGAGC	CTTGGTGGCT	ACATGCTGGG
Human	GCTGCTGCAG	CTGGAGCAGT	GGTAGGGGGC	CTCGGTGGCT	ACTTGCTGGG
Elk	GCTGCTGCAG	CTGGAGCAGT	GGTAGGGGGC	CTCGGTGGCT	ACATGCTGGG
Hamster	GGGGCGGTGG	TGGGGGGCCT	TGGGGGCTAC	ATGCTGGGGA	GCGCCATGAG

Dezhou don	GAGTGCCATG	AGCAGACCCC	TGATACACTT	TGGCAGTGAC	TATGAGGACC
Horse	GAGTGCCATG	AGCAGACCCC	TCATTCATTT	TGGCAATGAC	TATGAGGACC
Mink	GAGCGCCATG	AGCAGGCCCC	TCATTCATTT	TGGCAACGAC	TATGAGGACC
Bovine	AAGTGCCATG	AGCAGGCCTC	TTATACATTT	TGGCAATGAC	TATGAGGACC
Goat	AAGTGCCATG	AGCAGGCCTC	TTATACATTT	TGGCAATGAC	TATGAGGACC
Human	AAGTGCCATG	AGCAGGCCTC	TTATACATTT	TGGCAATGAC	TGTGAGGACC
Elk	AAGTGCCATG	AGCAGGCCTC	TTATACATTT	TGGCAATGAC	TATGAGGACC
Hamster	CAGGCCCATG	CTCCATTTTG	GCAATGACTG	GGAGGACCGC	TACTACCGTG

Dezhou don	GTTACTATCG	TGAAAACATG	TACCGTTACC	CCAACCAAGT	GTACTACAGG
Horse	GTTACTATCG	TGAAAACATG	TACCGTTACC	CCAACCAAGT	GTACTACAGG
Mink	GCTACTACCG	TGAGAACATG	TACCGCTACC	CCAACCAAGT	GTACTACAAG
Bovine	GTTACTATCG	TGAAAACATG	CACCGTTACC	CCAACCAAGT	GTACTACAGG
Goat	GTTACTATCG	TGAAAACATG	TACCGTTACC	CCAACCAAGT	GTACTACAGA
Human	GTTACTATCG	TGAAAACATG	TACCGTTACC	CCAACCAAGT	GTACTACAGG
Elk	GTTACTATCG	TGAAAACATG	TACCGTTACC	CCAACCAAGT	GTACTACAGG
Hamster	AAAACATGAA	CCGCTACCCT	AACCAAGTGT	ACTACCGGCC	AGTGGACCAG

Dezhou don	CCAGTGGATC	AGTACAGCAA	CCAGAACAGT	TTTGTGCATG	ACCGCGTCAA
Horse	CCGGTAAGTG	AGTACAGCAA	CCAGAAAAAC	TTTGTGCACG	ACTGCGTCAA
Mink	CCGGTGGATC	AGTACAGCAA	CCAGAACAAC	TTGGTGCATG	ACTGCGTCAA
Bovine	CCAGTGGATC	AGTATAGTAA	CCAGAACAAC	TTTGTGCATG	ACTGTGTCAA
Goat	CCAGTGGATC	AGTATAGTAA	CCAGAACAAC	TTTGTGCATG	ACTGTGTCAA
Human	TCAGTGGATC	AGTATAATAA	CCAGAGCACC	TTTGTGCATG	ACTGTGTCAA
Elk	CCAGTGGATC	AGTATAATAA	CCAGAACACC	TTTGTGCATG	ACTGTGTCAA
Hamster	TACAACAACC	AGAACAACTT	TGTGCACGAC	TGTGTCAATA	TCACGATCAA

Dezhou don	CATCACCGTC	AAGCAGCACA	CAGTGACCAC	GACCACCAAG	GGGGAGAACT
Horse	CATCACGGTC	AAGCAGCACA	CGGTCACCAC	CACCACCAAG	GGGGAGAACT
Mink	CATCACGGTC	AAGCAGCACA	CGGTGACCAC	CACCACCAAG	GGCGAGAACT
Bovine	CATCACAGTC	AAGGAACACA	CAGTCACCAC	CACCACCAAG	GGGGAGAACT
Goat	CATCACAGTC	AAGCAACACA	CAGTCACCAC	CACCACCAAG	GGGGAGAACT
Human	CATCACAGTC	AAGCAACACA	CAGT...CAC	CACCACCAAG	GGGGAGAACT
Elk	CATCACAGTC	AAGCAACACA	CAGTCACCAC	CACCACCAAG	GGGGAGAACT
Hamster	GCAGCATACA	GTCACCACCA	CCACCAAGGG	GGAGAACTTC	ACGGAGACCG

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Dezhou don    TCACCGAGAC CGACGTCAAG ATGATAGAGC GCGTGGTGGA ACAGATGTGC
Horse         TCACCGAGAC CGACGTCAAG ATCATGGAGC GCGTGGTGGA GCAGATGTGC
Mink          TCACGGAGAC CGACATGAAG ATCATGGAGC GCGTGGTGGA GCAGATGTGT
Bovine        TCACCGAAAC TGACATCAAG ATGATGGAGC GAGTGGTGGA GCAAATGTGC
Goat          TCACCGAAAC TGACATCAAG ATAATGGAGC GAGTGGTGGA GCAAATGTGC
Human         TCACCGAAAC TGACATCAAG ATGATGGAGC GAGTTGTGGA GCAAATGTGC
Elk           TCACCGAAAC TGACATCAAG ATGATGGAGC GAGTTGTGGA GCAAATGTGC
Hamster       ACGTCAAGAT GATGGAGCGC GTGGTGGAGC AGATGTGTGT CACCCAGTAT

Dezhou don    ATCACCCAGT ACCAGAAAGA GTACGAGGCA TACGCCCAAA GAGGGGCCAG
Horse         ATCACCCAGT ACCAGAAAGA GTACGAGGCT TTTCAACAAA GAGGGGCGAG
Mink          GTCACCCAGT ACCAGCAAGA GTCCGAGGCT TACTACCAGA GGGGGGCGAG
Bovine        ATCACCCAGT ACCAGAGAGA ATCCCAGGCT TATTACCAAC GAGGGGCAAG
Goat          ATCACCCAGT ACCAGAGAGA ATCCCAGGCT TATTACCAAA GGGGGGCAAG
Human         ATCACCCAGT ACCAGAGAGA ATCCGAGGCT TATTACCAAA GAGGGGCAAG
Elk           ATCACCCAGT ACCAGAGAGA ATCCGAGGCT TATTACCAAA GAGGGGCAAG
Hamster       CAGAAGGAGT CCCAGGCCTA CTACGACGG. ........AA GAAGATCCAG

Dezhou don    TGTGATCCTC TTCTCCTCCC CTCCTGTGAT CCTCCTCATC TCTTTCCTCC
Horse         CGTGGTCCTC TTCTCCTCCC CGCCTGTG.. .......... ..........
Mink          CGCCATCCTC TTCTCGCCCC CTCCCGTGAT CCTCCTCATC TCGCTGCTCA
Bovine        TGTGATCCTC TTCTCTTCCC CTCCTGTGAT CCTCCTCATC TCTTTCCTCA
Goat          TGTGATCCTC TTTTCTTCCC CTCCTGTGAT CCTCCTCATC TCTTTCCTCA
Human         TGTGATCCTC TTCTCCTCCC CTCCTGTGAT CCTCCTCGTC TCTTTCCTCA
Elk           TGTGATCCTC TTCTCCTCCC CTCCTGTGAT CCTCCTCATC TCTTTCCTCA
Hamster       CGCGGTGCTC TTCTCCTCTC CTCCTGTGAT CCTCCTCATT TCCTTCCTCA

Dezhou don    TTTTCCTCAT AGTGGG...C TGA....... .......... ..........
Horse         .......... .......... .......... .......... ..........
Mink          TTCTCCTGAT AGTGGG...A TGA....... .......... ..........
Bovine        TTTTTCTCAT AGTAGG...A TAGGGGCAAC CTTCCTGTTT TCAT......
Goat          TTTTTCTCAT AGTAGG...A TAGGGGCAAC CTTCCTGTTT TCATTATCTT
Human         TTTTTCTCAT AGTAGG...A TAGGGGCAAC CTTCCTGTTT TCATTAT...
Elk           TTTTTCTCAT AGTAGG...A TAGGGGCAAC CTTCCKGTTT TCATTATCTT
Hamster       TCTTCCTGAT AGTGGGATGA TGAGGGAAGC CTCCCTGCTT GTACTTCCTC

Dezhou don    .......... .......... .......... .......... ..........
Horse         .......... .......... .......... .......... ..........
Mink          .......... .......... .......... .......... ..........
Bovine        .......... .......... .......... .......... ..........
Goat          CTTAATCTTT GCCAGGTTGG GGGAGGGAGT GTCTACCTGC AGCCCTGTAG
Human         .......... .......... .......... .......... ..........
Elk           CT........ .......... .......... .......... ..........
Hamster       GTTCTTGTGC .......... .......... .......... ..........
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Dezhou don   .......... .......... ......
Horse        .......... .......... ......
Mink         .......... .......... ......
Bovine       .......... .......... ......
Goat         TGGTGGTGTC TCATTTCTTG CTTCTC
Human        .......... .......... ......
Elk          .......... .......... ......
Hamster      .......... .......... ......
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Fig. 2: The comparison of nucleic acid sequence of PrP gene between Dezhou donkey and seven other animals (accession number of GenBank are AF117317, U08952, AY367640, 326330, AY 458651, AY748455, M33958)

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Dezhou don MVKSHVGGWILVLFVATWSDVGLCKKRPKPGG.WNTGGSR    39
Horse      ................TWSDVGLCKKRPKPGGgWNTGGSR    24
Mink       MVKSHiGsWlLVLFVATWSDiGfCKKRPKPGGgWNTGGSR    40
Bovine     MVKSHiGsWILVLFVAmWSDVGLCKKRPKPGGgWNTGGSR    40
Goat       MVKSHiGsWILVLFVAmWSDVGLCKKRPKPGGgWNTGGSR    40
Human      MVKSHiGsWILVLFVAmWSDVGLCKKRPKPGGgWNTGGSR    40
Elk        MVKSHiGsWILVLFVAmWSDVGLCKKRPKPGGgWNTGGSR    40
Hamster    ..manlsyWlLaLFVATWtDVGLCKKRPKPGG.WNTGGSR    37

Dezhou don YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    79
Horse      YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    64
Mink       YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    80
Bovine     YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    80
Goat       YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    80
Human      YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    80
Elk        YPGQGSPGGNRYPPQGGGGWGQPHGGGWGQPHGGGWGQPH    80
Hamster    YPGQGSPGGNRYPPQGGGtWGQPHGGGWGQPHGGGWGQPH    77

Dezhou_don GGGWGQPHGGGGWGQGGGSHGQWNKPSKPKTNMKHVAGAA   119
Horse      GGGWGQPHGGGGWGQGG.SHGQWNKPSKPKTNMKHVAGAA   103
Mink       GGGWGQPHGGGGWGQGGGSHGQWgKPSKPKTNMKHVAGAA   120
Bovine     GGGWGQPHGGGGWGQGG.SHsQWNKPSKPKTNMKHVAGAA   119
Goat       GGGWGQPHGGGGWGQGG.SHsQWNKPSKPKTNMKHVAGAA   119
Human      GGGWGQPHGGGGWGQGG.tHsQWNKPSKPKTNMKHVAGAA   119
Elk        GGGWGQPHGGGGWGQGG.tHsQWNKPSKPKTNMKHVAGAA   119
Hamster    GGGWGQPHGGG.WGQGGGtHnQWNKPSKPKTNMKHVAGAA   116

Dezhou_don AAGAVVGGLGGYMLGSAMSRPLIHFGSDYEDRYYRENMYR   159
Horse      AAGAVVGGLGGYMLGSAMSRPLIHFGnDYEDRYYRENMYR   143
Mink       AAGAVVGGLGGYMLGSAMSRPLIHFGnDYEDRYYRENMYR   160
Bovine     AAGAVVGGLGGYMLGSAMSRPLIHFGnDYEDRYYRENMhR   159
Goat       AAGAVVGGLGGYMLGSAMSRPLIrFGnDYEDRYYRENMYR   159
Human      AAGAVVGGLGGYlLGSAMSRPLIHFGnDcEDRYYRENMYR   159
Elk        AAGAVVGGLGGYMLGSAMSRPLIHFGnDYEDRYYRENMYR   159
Hamster    AAGAVVGGLGGYMLGSAMSRPmlHFGnDwEDRYYRENMnR   156

Dezhou_don YPNQVYYRPVDQYSNQNSFVHDRVNITVKQHTVTTTTKGE   199
Horse      YPNQVYYRPVseYSNQknFVHDcVNITVKQHTVTTTTKGE   183
Mink       YPNQVYYkPVDQYSNQNnlVHDcVNITVKQHTVTTTTKGE   200
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Bovine     YPNQVYYRPVDQYSNQNnFVHDcVNITVKeHTVTTTTKGE     199
Goat       YPNQVYYRPVDQYSNQNnFVHDcVNITVKQHTVTTTTKGE     199
Human      YPNQVYYRsVDQYnNQstFVHDcVNITVKQHTVTTT.KGE     198
Elk        YPNQVYYRPVDQYnNQNtFVHDcVNITVKQHTVTTTTKGE     199
Hamster    YPNQVYYRPVDQYnNQNnFVHDcVNITiKQHTVTTTTKGE     196

Dezhou_don NFTETDVKMIERVVEQMCITQYQKEYEAYAQ.RGASVILF     238
Horse      NFTETDVKimERVVEQMCITQYQKEYEAfqQ.RGASVvLF     222
Mink       NFTETDmKimERVVEQMCvTQYQqEsEAYyQ.RGASaILF     239
Bovine     NFTETDiKMmERVVEQMCITQYQrEsqAYyQ.RGASVILF     238
Goat       NFTETDiKimEgVVEQMCITQYQrEsqAYyQ.RGASVILF     238
Human      NFTETDiKMmERVVEQMCITQYQrEsEAYyQ.RGASVILF     237
Elk        NFTETDiKMmERVVEQMCITQYQrEsEAYyQ.RGASVILF     238
Hamster    NFTETDVKMmERVVEQMCvTQYQKEsqAYydgRrsSavLF     236

Dezhou_don SSPPVILLISFLLFLIVG                           256
Horse      SSPPV.............                           227
Mink       SpPPVILLISlLilLIVG                           257
Bovine     SSPPVILLISFLiFLIVG                           256
Goat       SpPPVILLISFLiFLIVG                           256
Human      SSPPVILLvSFLiFLIVG                           255
Elk        SSPPVILLISFLiFLIVG                           256
Hamster    SSPPVILLISFLiFLIVG                           254
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Fig. 3: The comparison of amino acid sequence of PrP between Dezhou donkey and seven other animals. (Accession numbers of GenBank are AF117317, U08952, AY367640, AY326330, AY 458651, AY748455 and M33958)

encompassing a N-terminal nonapeptides (PQGGGGWGQ), four repeats of the octapeptides (PHGGGWGQ) and a C-terminal nonapeptides (PHGGGGWGQ). Amino acids 97~112 is a functional domain controlling the topology structure of the PrP^c; 113~135 is an α-helix; 157~177 is a hydrophilic helix; 234~256AA is a hydrophobic signal region (Fig. 3).

Comparing with horse, bovine, goat, deer, mouse, human being and mink, Dezhou donkey was unique in its PrP amino acids at the following positions: there was no Gly at position 32, nor in zebra (Wopfner *et al*., 1997) and there was a Gly at position 97; the positions 146, 182, 209, 225 and 229 were Ser, Arg, Iso, Tyr, Ala respectively in Dezhou donkey but Asp, Cys, Met, Ser and Tyr in other animals analyzed. Overall, the PrP of all species analyzed mainly varied in three regions: 90~103, 167~169 and 206~230. It has been reported that amino acid sites 90~130 are corresponding to the species barrier and 108~189 are related to incubation. Within these regions, Gly at position 100 in Dezhou donkey and horse was substituted with Ser in bovine, goat, human and elk. Outside these regions, positions 225 (Tyr) and 229 (Ala) were unique to Dezhou donkey and horse as compared to other species (Ser and Tyr). While the latter species but not the *equus* have been reported to be infected by prion disease, it is not known if these residues in particular are implicated in the species barrier in *equus*.

Recent studies with bovine transgenic mice suggested that amino acid residues 184, 186, 203 and 205 form an epitope that is involved in the control of the species barrier and that amino acids 167, 171, 214 and 218 form an epitope to interact protein X that has been found to participate in the PrP^{sc} formation(Kaneko *et al*., 1997; Brown *et al*., 1997). However, analysis of these sites showed no variation of amino acids between Dezhou donkey and the rest of the animals analyzed. As for polymorphism at 136, 154 and 171 amino acid sites that has been reported to be pertinent to impressionable of scrapie (Eghiaian *et al*., 2004; Mallucci *et al*., 2003), in which the genotype A136R154R171 induces resistance to scrapie but A136R154Q171 is intermediately sensitive and V136R154Q171 is sensitive to scrapie, the genotype of donkey is ARQ and may therefore be intermediately sensitive to scrapie. However, how these differences influence the conversion of PrP^{c} to PrP^{sc} are yet to be illuminated.

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