Breeding of Anti-diarrhea Gene in Local and Exotic Pig Breeds in Guizhou Province

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Abstract [**Objectives**] This study was conducted to breed special pig breeds resistant to diarrhea by using modern biotechnology. [**Methods**] From Guizhou local breeds, such as Nuogu pigs, Kele pig, Yorkshire pigs and Duroc pigs, 190 samples were collected for the analysis of anti-diarrhea gene. [**Results**] The anti-diarrhea genotype frequency of Kele pigs was 70.00%, which was higher than that of Nuogu pigs (67.37%) and Yorkshire pigs (Yorkshire pigs and Duroc pigs) (50.59%). The favorable anti-diarrhea gene of all Nuogu pigs, Kele pigs, and Yorkshire pigs and Duroc pigs was G, with gene frequencies of 0.735 5, 0.836 8 and 0.850 0, respectively, and the frequencies of allele A were 0.264 5, 0.163 2 and 0.150 0, respectively. In the process of generation selection, combination selection of GG ♂ × GG ♀, GG ♂ × GA ♀, GA ♂ × GG ♀ and GA ♂ × GA ♀ was conducted, and GG individuals were selected while gradually phasing out GA and AA individuals. The anti-diarrhea genotypes of 98 pigs in the offspring were tested, and it was found that the frequency of genotype GG was greatly improved, and the frequencies in Nuogu pigs, Kele pigs, Yorkshire pigs and Duroc pigs were increased to 73.91%, 81.82%, 85.25% and 66.67% respectively, thus forming a special anti-diarrhea breed. [**Conclusions**] This study provides a basis for selecting excellent breeding pigs, establishing core populations and screening resistance genes in the core populations and their offspring.

Key words Pig; Anti-diarrhea; *MUC13* gene; Generation selection; New breed

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Diarrhea of suckling piglets is a common and frequently-occurring disease in large-scale pig farms, which seriously affects the survival rate of piglets and causes great economic losses[1-2]. Enterotoxigenic *Escherichia coli* (ETEC) is one of the important pathogens causing severe diarrhea in humans and pigs[3]. Studies have shown that *MUC13* gene located on pig chromosome 13 (SSC13q41-q44) plays a key role in ETEC infection, and has the genetic basis of susceptibility or resistance, which is an autosomal recessive monogenic inheritable character[2,4]. *MUC13* gene is mainly expressed on the epithelial surface of gastrointestinal tract, and it shows a strong linkage imbalance with ETEC F4ab/ac receptor site, which provides a useful molecular marker for selecting individuals resistant to ETEC F4ab/ac in pig breeding[5-6]. Meanwhile, *MUC13* gene can also inhibit the invasion of pathogenic bacteria such as *Salmonella typhimurium* and maintain the integrity of the barrier[7]. Therefore, the anti-diarrhea genotype can be retained in the process of generation selection by screening anti-diarrhea genes using modern disease-resistant breeding techniques, so as to cultivate new anti-diarrhea special strains and improve the economic benefits of pig farms.

In this study, the ETEC F4ac receptor gene *MUC13*, which has an important influence on piglet diarrhea, was selected as the object, and the imported American Yorkshire with 6 lineages, Canadian Yorkshire with 3 lineages, Duroc with 3 lineages, and Guizhou local breeds of Nuogu pigs and Kele pigs were subjected to pedigree breeding to strengthen generation selection. Excellent breeding pigs were selected through production performance and genetic evaluation to establish core populations, and the resistance gene was screened in the core populations and their offspring.

Materials and Methods

Experimental pig breeds

Guizhou local breeds Nuogu Pigs and Kele Pigs, and Yorkshire Pigs and Duroc Pigs from Nayong Demonstration Farm in Bijie City, Guizhou Province.

Methods

DNA extraction First, 2 – 3 g of ear tissue was collected, added in an senzyme-free tube and stored at –20 ℃. Genomic DNA was extracted by tissue genomic DNA extraction kit of Tiangen Biotech (Beijing) Co., Ltd. and used as a template for PCR detection.

PCR amplification of anti-diarrhea gene According to the sequence of pig *MUC13* gene published by GenBank, four specific primers (Table 1) were designed and synthesized by Invitrogen Trading (Shanghai) Co., Ltd.

Table 1 Primer sequences

Primer	Primer sequence (5'→3')	Anticipated fragment//bp	Annealing temperature//℃
F1	ACATTTCAGAGTCTGAGGGATG	83	60
R1	CTCCTCACCAGCTCCTTAGC		
F2	GAGAGACCAAACCCACAGAT	173	63
R2	GGCCTATAGCCAAAATTCAT		

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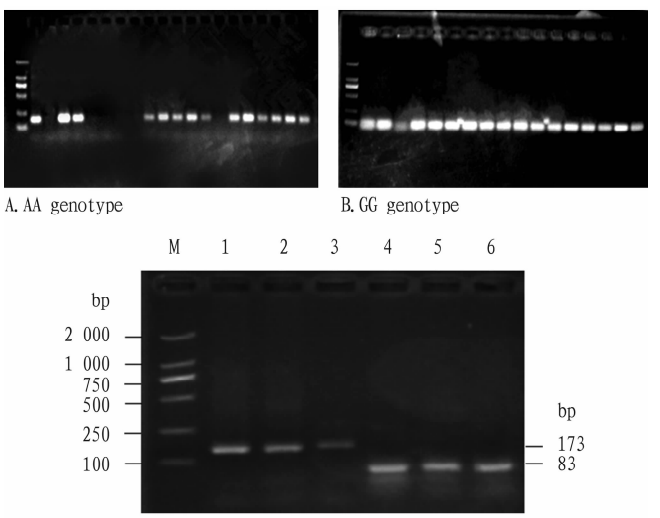
The total amount of PCR reaction system was 10 μl, including 5.0 μl of 2 × PCR mixed solution, 0.5 μl of primers (10 pmol/L), 1.0 μl of genomic DNA (100 ng/μl) and 10 μl of double distilled water. The amplification was started with pre-denaturation at 95 ℃ for 5 min, followed by 30 cycles of denaturation at 95 ℃ for 30 s, annealing at 60 ℃ or 63 ℃ for 30 s and extension at 72 ℃ for 45 s, completed by extension at 72 ℃ for 10 min, and the products were stored at 4 ℃. The amplification products were detected by 2% agarose gel electrophoresis.

Results and Analysis

Detection results of anti-diarrhea genotypes in pigs

In the process of detection, the 173 bp band was defined as gene A and the 83 bp band was defined as gene G. If a sample had only the 173 bp band, it should be of AA genotype; if it had only the 83 bp band, it should be of GG genotype; and when two bands appeared at the same time, it should be a heterozygous AG genotype (Fig. 1).



Allele G, resistance gene; allele A, susceptibility gene; AA and GA diarrhea-susceptible individuals; GG diarrhea-resistant individuals. 1/2, AA type; 3/4, AG type; and 5/6, GG type.

Fig. 1 Detection bands of pig MUC13 genotypes

Cloning and sequencing

The 173 and 83 bp bands obtained by PCR were recovered by gel cutting and connected to T vector for cloning and sequencing. Sequencing results confirmed that the amplified fragments were *MUC13* gene, located in intron 7, and the corresponding base was G or A (Fig. 2).

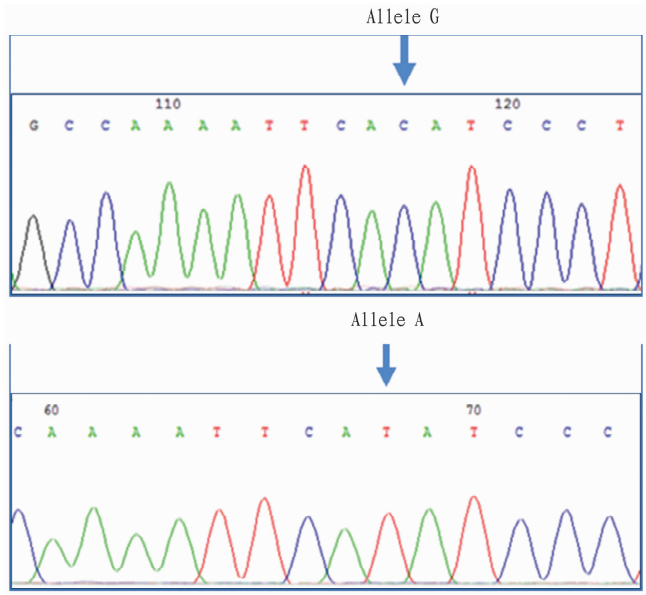


Fig. 2 Sequencing maps of alleles G and A

After gel recovery, the fragments obtained by primer amplification were compared with known sequences. The results showed that fragment 1 and fragment 2 only differed in the base at locus 119, and G/A variation occurred (Fig. 3), which confirmed that this locus was SNP located in intron 7 of *MUC13* gene.

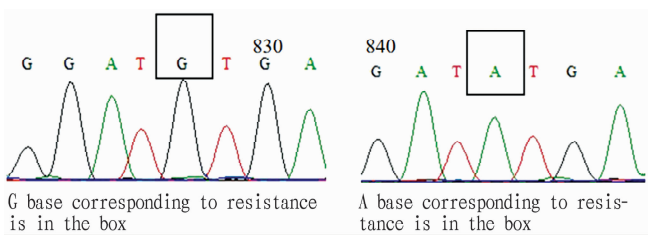


Fig. 3 Base determination results of G119A locus of *MUC13* gene

Genotype frequency and allele frequency of *MUC13* gene in core populations of pig breeds

A total of 190 ear tissue samples were collected from the core populations of different pig breeds in Nayong Demonstration Farm in Bijie City, Guizhou Province for anti-diarrhea gene testing. These included 85 Yorkshire pigs (covering 7 American lineages and 3 Canadian lineages), 95 Guizhou local breed Nuogu pigs (covering 3 families) and 10 Kele pigs (covering 3 families), as shown in Table 2.

Table 2 Genotype frequency and allele frequency of *MUC13* gene in core populations of pig breeds

Breed	Number of sample	Genotype frequency//%			Gene frequency//%	
		GG	AG	AA	G	A
Yorkshire pigs	85	50.59 (43/85)	45.88 (39/85)	3.53 (3/85)	73.53 (125/170)	26.47 (45/170)
Nuogu pigs	95	67.37 (64/95)	32.63 (31/95)	0	83.68 (159/190)	16.32 (31/190)
Kele pigs	10	70.05 (7/10)	30.00 (3/10)	0	85.00 (17/20)	15.00 (3/20)
Total	190	60.00 (114/190)	38.42 (73/190)	3.53 (3/85)	79.21 (301/380)	20.79 (79/380)

It can be seen that the frequency of anti-diarrhea genotype GG in the core population of Yorkshire pigs was 50.59% (43/85), which was significantly lower than 67.37% (64/95) in the core population of Nuogu pigs and 70.0% (7/10) in the core population of Kele Pigs. Meanwhile, through chi-square test, it was found that there was a significant difference in the frequency of MUC13 allele between Yorkshire and Nuogu pigs ($P < 0.05$), and the frequency of anti-diarrhea genotype GG in Nuogu pigs was significantly higher than that in Yorkshire pigs. There were also a significant difference in the frequency of MUC13 allele between Yorkshire and Kele pigs ($P < 0.05$). However, no significant difference was observed in the frequency of MUC13 allele between Nuogu pig and Kele pig ($P > 0.05$).

Establishing special anti-diarrhea populations and carrying out offspring testing

Selective breeding based on *MUC13* gene aims to improve the anti-diarrhea ability of pigs and improve the production performance and economic benefits of pig industry. The above-mentioned core populations of American Yorkshire with 6 lineages, Canadian Yorkshire with 3 lineages and Duroc with 3 lineages, as well as Guizhou local breeds of Nuogu pigs and Kele pigs, were purebred, and special anti-diarrhea populations were established. In the process of generation selection, the combinations of GG ♂ × GG ♀, GG ♂ × GA ♀, GA ♂ × GG ♀ and GA ♂ × GA ♀ were selected, and the anti-diarrhea genotypes of their offspring were detected and screened. During the process, we collected 98 ear tissue samples, including 23 Guizhou local breed Nuogu pigs and 11 Kele pigs, 61 Yorkshire pigs and 3 Duroc pigs, and made genotype analysis, as shown in Table 3.

The results showed that the anti-diarrhea gene of 25 F_1 and F_2 reserve pigs selected randomly by GG ♂ × GG ♀ were all type GG, which has the ability of stable inheritance. The frequency distribution of genotype GG in different breeds of pigs was as follows: Yorkshire 85.25% (52/61), Kele 81.82% (9/11), Nuogu 73.91% (17/23) and Duroc 66.67% (2/3). From high to low, the frequency of anti-diarrhea gene G was 90.91% (20/22) in Kele pigs, 90.16% (110/122) in Yorkshire pigs, 86.96% (40/46) in Nuogu pigs and 83.33% (5/6) in Duroc pigs.

Table 3 Distribution of genotype and gene frequency of MUC13 in offspring of breeding pigs in generation selection

Breed	Number of detected pigs//pigs	Genotype frequency//%			Gene frequency//%	
		GG	GA	AA	G	A
Yorkshire pigs	61	85.25 (52/61)	9.84 (6/61)	4.92 (3/61)	90.16 (110/122)	9.84 (12/122)
Nuogu pigs	23	73.91 (17/23)	26.09 (6/23)	0	86.96 (40/46)	13.04 (6/46)
Kele pigs	11	81.82 (9/11)	18.18 (2/11)	0	90.91 (20/22)	9.09 (2/22)
Duroc pigs	3	66.67 (2/3)	33.33 (1/3)	0	83.33 (5/6)	16.67 (1/6)
Total	98	81.63 (80/98)	15.31 (15/98)	4.92	89.29 (175/196)	10.71 (21/196)

Conclusions and Discussion

Distribution of MUC13 genotypes in Yorkshire, Nuogu and Kele pigs after breeding

After molecular breeding, Yorkshire pigs, Nuogu pigs and Kele pigs showed differences in MUC13 genotype. Among 146 Yorkshire pigs, 65.07% (95/146) were homozygous GG, 30.82% (45/146) were heterozygous GA and 4.11% (6/146) were homozygous AA. Among 118 Nuogu pigs, genotype GG accounted for 68.64% (81/118), genotype GA accounted for 31.36% (37/118), and there was no homozygous genotype AA. Among 21 Kele pigs, genotype GG accounted for 76.19% (16/21), genotype GA was 23.81% (5/21), and there was no homozygous genotype AA. In addition, two anti-diarrhea Duroc boars, which could be used as resistant male parents for cross breeding, were selected. The above results showed that: ① the receptor gene *MUC13* of enterotoxigenic *E. coli* (ETEC) F4ac was related to pig breeds, and there were significant differences in the distribution of anti-diarrhea gene among different breeds. Meanwhile, it was found that under the same feeding mode, management and environmental conditions, the incidence rates of diarrhea in different breeds of piglets were different. ② Through the assisted breeding of anti-diarrhea genes, new special anti-diarrhea breeds could be effectively established and optimized. ③ Breed differences should be considered in the breeding process to improve diarrhea resistance.

Breeding of new anti-diarrhea purebreds of GG genotype

In the process of purebred breeding, it is necessary to take into account the factors such as pedigree, body shape and production performance, and stabilize the genetic basis of resistance through multi-generation homozygous breeding. Different genotype combinations, such as GG ♂ × GG ♀, GG ♂ × GA ♀, GA ♂ × GG ♀, and GA ♂ × GA ♀, can be used. It could be determined that the offspring of individuals with GG ♂ × GG ♀ were all anti-diarrhea genotype GG with stable inheritance, and had the ability to stably inherit favorable genotype GG. Except GG ♂ × GG ♀, the offspring of other three combinations all had a certain proportion of anti-diarrhea genotype. Therefore, it is necessary to increase the selection of GG ♂ × GG ♀ individuals, eliminate individuals of AA genptype and gradually eliminate individuals of GA genotype, so as to expand excellent populations of anti-diarrhea pigs. Meanwhile, the size of the basic population affects the difficulty and time of breeding, and a small scale means high difficulty and longer time. Through years of breeding, Guizhou Provincial Breeding Livestock and Poultry Germplasm Testing Center has developed four special resistant breeds, including American and Canadian Yorkshire Pigs, Duroc Pigs, Nuogu Pigs and Kele Pigs. They provide excellent anti-diarrhea parents for binary and ternary hybridization and the utilization of local breeds, reduce the diarrhea rate and mortality of piglets and improve the economic benefits of pig farms.

Comprehensive epidemic prevention measures for diarrhea symptoms

There are many factors affecting diarrhea in suckling piglets, including environmental sanitation, temperature fluctuation, heat preservation and ventilation conditions in the house[8-9]. Even piglets with anti-diarrhea genotype GG may have diarrhea. Therefore, it is necessary to establish and implement complete comprehensive epidemic prevention measures in pig farms, such as vaccine immunization and drug treatment for susceptible sows, so as to effectively prevent piglet diarrhea and improve the survival rate[10].

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