Polymorphisms associated with resistance to infectious diseases in different breeds of pigs of the Belgorod Region of Russia

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Abstract. Using the PCR-RFLP method, we genotyped pigs for the genes GBP1 (mutation c. [10A>G; 11A>G]), FUT1 (mutation G307A) and MUC4(A243G) associated with resistance to infectious diseases. A total of 188 boars of four breeds (Duroc, Large White, Landrace and Yorkshire) from the Belgorod Region of Russian Federation were studied. The highest frequency of favorable genotype AG for the polymorphism GBP1E2, which is resistant to pig respiratory syndrome, was characterized by the Duroc breed (0.38). The highest frequency of *Eischerichia coli* F18-resistant AA genotype was observed in the Landrace population (0.19). And in terms of resistance to *E. coli* with fibrium type F4 (K 88), the highest frequency of favorable genotype GG is characterized by the breed Large White (0,23). In general, it can be noted that genetic resistance in the studied boars is observed predominantly only to one infectious disease.

1 Introduction

Modern pig breeding faces the problem of active spread of infectious diseases and the need to develop a set of methods to control them [1]. A promising cost-effective way to control infectious diseases in pigs is marker-mediated selection by disease resistance genes [2, 3]. To date, resistance genes and associated markers for many common infectious diseases of pigs have been described.

One of the most common diseases that cause significant economic damage in pig production is porcine reproductive and respiratory syndrome (PRRS), the causative agent of which is RNA-containing virus [4]. This virus damages mucosal surfaces and disrupts the cellular immune response, leads to infertility, increased number of late-term abortions, as well as sow death, young deaths, reduced meat and fattening productivity, and decreased meat sanitary quality [5, 6, 7]. The c. polymorphism [10A>G; 11A>G] of the GBP1 (GBP1E2) gene can be treated as a genetic marker of resistance to PRRS [8]. A number of publications have shown that heterozygous AG pigs showed a lower viral load in serum and lungs, higher average daily weight gain, demonstrated a larger T-cell population, and more alpha interferon in serum than pigs with AA and GG genotypes [9, 10].

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Eischerichia coli F18 bacteria are the main pathogens that cause post-weaning edema and diarrhea in piglets, and the alpha-1-fucosyltransferase (FUT1) gene has been identified as a candidate gene for the control of F18 receptor expression [11, 12, 13].

The G307A (*BstHHI*) polymorphism in the FUT1 gene has been found to be associated with resistance to *E. coli* F18 adhesion in the small intestine [14]. *E. coli* resistant is the AA genotype found in Western breed populations [11, 15].

Another gene involved in the interaction of enterotoxigenic E. coli bacteria with fibrium type F4 (K 88) and intestinal receptors in piglets is the MUC4 gene [16, 17]. Analysis of the A243G (*BstHHI*) polymorphism in intron 17 shows the association of the GG genotype with resistance to colibacillosis, increased antitumor ability, and higher growth rate of groups during the fattening period [18]. In addition, MUC4 is considered as a marker of reproductive performance of pigs related to the piglets number at birth and multiple births [18].

Thus, the genotyping of pigs by markers associated with resistance to infectious diseases allows the selection of animals and the formation of disease-resistant livestock.

The aim of this study is to estimate the frequency of alleles and genotypes for polymorphisms G307A of FUT1 gene, A243G of MUC4 gene and GBP1E2 in Yorkshire, Landrace, Large White and Duroc pigs in farms of Belgorod region of Russian Federation.

2 Materials and methods

The objects of the research were boars of the main pig breeds from farms in the Belgorod region of Russian Federation: Yorkshire (47 animals), Landrace (47 animals), Large White (47 animals) and Duroc (47 animals),

Genomic DNA was extracted from alcoholized ear pluckings applying a DNA-Extran-2 reagent kit (SINTOL, Russian Federation) following the protocol. Genotyping of pigs was performed by PCR-RFLP method. The volume of PCR mixture was 20 µl, containing 20 ng of genomic DNA, 10 mM Tris-HCl (*pH*=8.3), 50 mM KCl, 4 mM MgCl2, 0.25 mM dNTP, 0.5 µM of each primer, and 1 unit Taq DNA polymerase. The primers are shown in Table 1. The amplification mode is common for all three markers: 94°C - 5 min, 35 cycles [94°C - 20 s, 60°C - 20 s, 72°C - 40 s], 72°C - 5 min 1. The reaction was performed on a Veriti amplifier (Applied Biosystems, USA). A 10 µl PCR product was hydrolyzed with 5 units of the appropriate *Bse21I* or *BstHHI* restrictase (SibEnzyme, Russian Federation) for 16 hours (Table 1). The hydrolysis products were separated by horizontal electrophoresis in 2% agarose gel (Mini-Sub Cell GT chamber, BioRad, USA). To identify DNA sequences, the gel blocks were stained with with ethidium bromide (0.5 µg/ml) and visualized on a UV-transilluminator (Figure 1).

Microsoft Excel and GenAlEx software were used for analysis of the data obtained.

3 Results and discussion

Obtained data shows that breeds of pigs in the Belgorod region of Russian Federation are characterized by different frequencies of genotypes and alleles according to the GBP1E2 polymorphism (Table 2). Duroc and Landrace breeds are characterized by more equalized allele frequencies and, accordingly, a higher frequency of the most valuable genotype AG. The proportion of PRRS-resistant animals in these breeds reaches 38 and 30%, respectively. On the other hand, the lowest proportion of heterozygotes is noted for Yorkshire (2%) and Large White (6%) breeds, which indicates that these breeds are most vulnerable to PRRS and that additional measures are needed to reduce the prevalence of the virus in their housing areas.

As might be expected, the FUT1 G307A polymorphism revealed a favorable allele A in all the studied breeds. Its frequency ranges from 0.22-0.36, which is somewhat higher than the previously obtained data in other populations of these breeds [12, 14, 19, 20, 21].

Table 1. Conditions for PCR-RFLP.

Gene /SNP	Primers	PCR product	Enzyme	Hydrolysis products
GBP1E2	GBPIE2F GGATAACACTTCGGTAACTTGC, GBPIE2R GAAGGGGAAACTGAGACACAAT [8]	587 bp	*Bse21I*	*GG* – 385and 202 bp; *AA* – 587 bp; *AG* – 587, 385 and 202 bp
FUT G307A	FUTIF CGCCACCTCTGTCTGACCTT FUTIR AAGGAGCGTGCCTGTCTACCT	400 bp	*BstHHI*	*GG* – 290, 87 and 23 bp; *AA* – 377 and 23 bp; *AG* – 377, 290, 87 and 23 bp
MUC4 A243G	MUC4F CAGGATGCCCAATGGCTCTAC MUC4R CCCCGAAGTTGTGAAAGGAAG [16]	538 bp	*BstHHI*	*GG* – 295 and 243 bp; *AA* – 538 bp; *AG* – 538, 295 and 243 bp

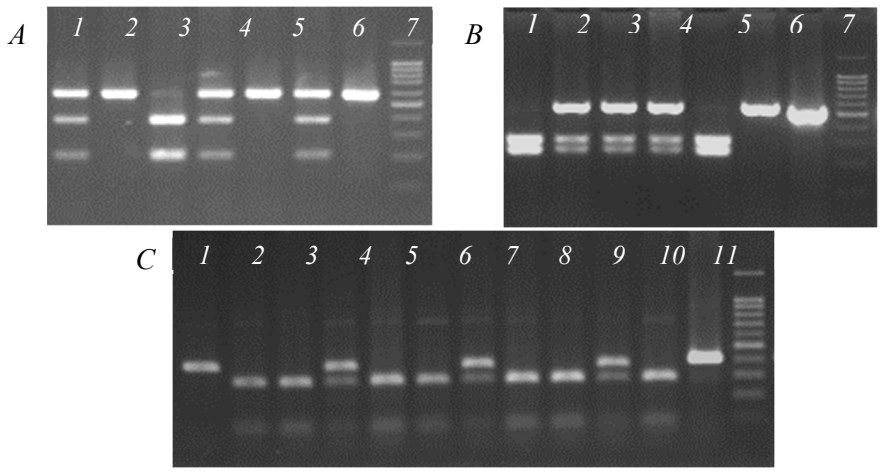

Fig. 1. Electrophoresis result of PCR products after hydrolysis: A - 587 bp fragment (GBP1E2) by Bse21I site. Cells #4,7 - AA genotype, #3,5,8 - AG genotype, #6 - GG genotype, #2 - PCR product, #1 - 100+ length marker; B - 538 bp fragment (MUC4 A243G) by BstHHI site. Cells #1,5 - GG genotype, #2,3,4 - AG genotype, #6 - AA genotype, #7 - PCR product, #8 - 100+ length marker; C - 400 bp fragment (FUT G307A) by BstHHI site. Cells #1 - AA genotype, #2,3,5,6,8,9,11 - GG genotype, #4,7,10 - AG genotype, #12 - PCR product, #13 - 100+ length marker.

The highest fraction of resistant animals with AA genotype is observed in the Landrace population (19%), and the lowest - Durocs (6%). It is noteworthy that the observed frequency of homozygotes in Landraces is one of the highest among the published data, which may indicate that breeding work on the selection of animals resistant to E. coli infection with F4 fibrium type.

According to the MUC A243G polymorphism the highest frequency of the stable GG genotype is typical for the Large White breed (23%), and the lowest - for Landrace (2%). In

general, the values obtained correspond to the literature data on the distribution of allele and genotype frequencies in pig breeds [18, 21]. As in the case of resistance to PRRS, such a significant variation in the proportion of resistant animals in different breeds requires differential measures to reduce the prevalence of infection in the animal housing areas of different breeds.

Table 2. Frequencies of alleles and genotypes for the studied polymorphisms in boars of different breeds.

Marker	Allele/genotype	Duroc	Large White	Landrace	Yorkshire
GBP1E2	A	0.766	0.968	0.830	0.989
	G	0.234	0.032	0.170	0.011
	AA	0.574	0.936	0.681	0.979
	AG	0.384	0.064	0.298	0.021
	GG	0.042	0.000	0.021	0.000
FUT G307A	A	0.223	0.362	0.319	0.340
	G	0.777	0.638	0.681	0.660
	AA	0.064	0.149	0.191	0.149
	AG	0.319	0.426	0.256	0.383
	GG	0.617	0.425	0.553	0.468
MUC A243G	A	0.574	0.521	0.894	0.596
	G	0.426	0.479	0.106	0.404
	AA	0.255	0.277	0.809	0.362
	AG	0.638	0.489	0.170	0.468
	GG	0.106	0.234	0.021	0.170

Table 3. Results of χ^2 test for the correspondence of genotype frequencies to the Hardy-Weinberg distribution in pig breeds and inbreeding coefficient (F).

Breed	GBP1E2		FUT G307A		MUC A243G	
	χ^2	*F*	χ^2	*F*	χ^2	*F*
Duroc	0.218 (*ns*)	-0.068	0.303 (*ns*)	0.080	4.388 (*)	-0.306
Large White	0.051 (*ns*)	-0.033	0.289 (*ns*)	0.078	0.018 (*ns*)	0.020
Landrace	0.140 (*ns*)	-0.054	7.997 (**)	0.413	0.516 (*ns*)	0.105
Yorkshire	0.005 (*ns*)	-0.011	1.018 (*ns*)	0.147	0.037 (*ns*)	0.028
On average by region	0.231 (*ns*)	-0.042±0.013	7.038 (**)	0.180±0.079	0.222 (*ns*)	-0.038±0.091
ns – no credible differences						
* – P<0.05						
** – P<0.01						

For most of the studied breeds, the empirical genotype frequencies do not differ significantly (P>0.05) from the theoretically expected Hardy-Weinberg distribution (Table

3). The exception is the FUT G307A polymorphism in Landraces, caused by an excess of unfavorable homozygous genotype GG, as well as MUC A243G in Durocs. Here, there is an excess also of the unfavorable heterozygous genotype AG. Thus, there is a deficit of favorable genotypes in the above breeds by markers of resistance to infections. Most likely, this picture is caused by breeding work on other economically important traits.

Thus, different breeds of pigs show different degrees of genetic resistance to infectious diseases. For example, Durocs are the most resistant to PRRS, but they are the most susceptible to E. coli F18. Conversely, the most resistant breed to E. coli F18 is the Large White, but at the same time it is highly susceptible to PRRS. Therefore, we can conclude that no breed equally resistant to several diseases or simultaneously susceptible to all diseases is recorded among the studied animals.

The conclusion is also consistent with the fact that no animal with a genotype resistant to three analyzed diseases at once was found among the 188 studied animals. At the same time, only 9 out of 188 animals have genotypes resistant to two diseases at once.

4 Conclusion

Thus, the analysis of allele frequencies genotypes and of boars of Yorkshire, Landrace, Large White and Duroc breeds from farms of Belgorod region of Russian Federation by polymorphisms GBP1E2 of gene GBP1E, G307A of gene FUT1 and A243G of gene MUC4 was carried out. The results of the study match data from other sources. Duroc pigs were the most resistant to E. coli F18, Landrace pigs - to E. coli F18, and Large White pigs - to E. coli F4. At the same time, the situation of animal resistance to only one type of infectious disease is observed.

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