Reduced antibiotic use in piglets: implementation of a breeding program for *E.Coli* F4ab/ac resistant pigs

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Introduction

Enterotoxigenic *E.*coli (ETEC) infection is one of the major causes of diarrhoea and death among neonatal and young piglets which will cause severe economic losses as well (Melkebeek, Goddeeris, & Cox, 2013). The bacteria carrying different F4 fimbriae are able to colonize the small intestine by adhering to specific receptors (*F4bcR*) on the brush borders of enterocytes, and they subsequently produce toxins which will cause diarrhoea (Nataro & Kaper, 1998). F4 fimbriae exist in three antigenic variants – F4ab, F4ac and F4ad – that varies in their receptor specificities (Frydendahl, 2002). Resistant or susceptible phenotypes for ETEC F4ab/F4ac are inherited as an autosomal recessive monogenetic trait in pigs. Selection of genetically F4-resistant pigs is a sustainable and suitable alternative to decrease animal loss and antibiotic use due to diarrhoea. An absolute or relative disease resistant pig could diminish the severity of illness, decrease pathogen proliferation and excretion, and in consequence decrease the infection pressure on healthy pigs. Previous studies have localised the ETEC F4ab/F4ac receptor gene (*F4bcR*) on pig chromosome 13 (SSC13q41-q44) in the vicinity of exon 2 of the *MUC13* gene (Python et al., 2002; Rampoldi et al., 2011; Ren et al., 2012). Various markers were used for genotyping *F4bcR* and indeed Two flanking markers (*CHCF1* and *CHCF2*) with high linkage disequilibrium (LD) to the *F4bcR* were detected and found effectively identify genetically F4-resistant pigs (Figure 1).

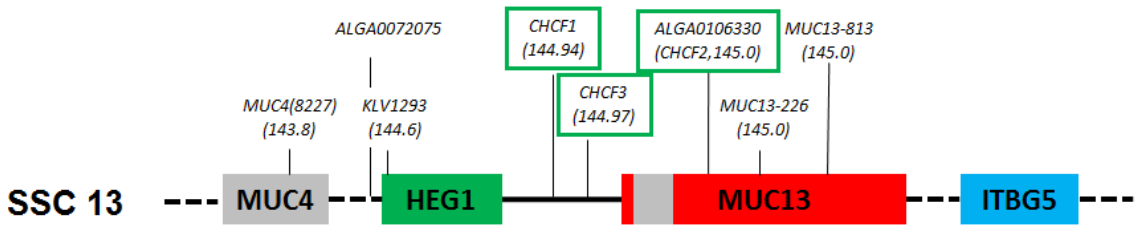

Figure 1: Section of the pig chromosome 13 (SSC13) including annotated genes and markers. The location of the receptor locus for the adhesion of *E. coli* F4ab/ac (*F4bcR*) is most likely between the markers CHCF1 and CHCF2).

Materials and Methods

Estimation of the resistant allele effect on production traits

Ear biopsies of 530 Swiss Large White pigs from the breeding test station were taken and DNA was extracted to be genotyped with markers *CHC*F1 and *CHCF2* by using a Kompetitive Allele Specific PCR (KASP) assay. A mixed model with a fixed genotype effect and a random sire effect was used to estimate the effect of the resistant alleles on 17 important production traits using the statistic program R.

12 recombinant boars (genotype results of *CHCF1* and *CHCF2* are different) were typed with additional Markers (Figure 1) and the *E.coli* F4 microscopic adhesion test (MAT) was performed in order to determine the presence or absence of F4bcR on the brush border of small intestinal enterocytes.

Development of *E. coli* F4ac in resistant and susceptible piglets

50 resistant and 44 susceptible piglets were infected orally with a 5ml ETEC (strain *35HI,* F4ac, LT+, STb+) suspension of 10^8 CFU/ml at day 4 after weaning. Faeces samples of 21 resistant and 18 susceptible piglets without diet additions were collected from the day before infection (day 0) to day 7 after infection. The colony forming units (CFU per µg of DNA) were determined by counting colonies on EMBRif50 petri plate from different bacteria dilutions.

Results and Discussion

The feasibility of a breeding program to select for *E.coli* F4 resistant animals depends on the frequency of the resistance allele in the population and its effect on important economic traits. Genotyping result of 1429 Swiss Large White with KASP by *CHCF1* and *CHCF2* show that around two-thirds F4bcR susceptible pigs (168 homozygous susceptible and 720 or 721 heterozygous) and one-third F4bcR resistant pigs (541 or 540 resistant). In only one sample we found a recombination between *CHCF1* and *CHCF2* (< 0.07%) which demonstrated that the DNA-based test to distinguish *F4bcR* genotypes is practical. The allele frequency of the resistant alleles is around 63%, which is very advantageous for breeding resistant animals. Various markers in the interval *MUC4-MUC13* (Figure 1) and MAT test were used to characterize *CHCF1/CHCF2* recombinant pigs in Swiss Landrace, Duroc and Piétrain pigs. In Swiss Large White, both *CHCF1* and *CHCF2* were comparable however in the other breeds *CHCF1* and *CHCF3* were much closer linked to *F4bcR* than *CHCF2*. Preliminary analysis on 530 Swiss Large White from the breeding test station showed that a significant difference among *F4bcR* genotypes only in intramuscular fat (IMF) content of the longissimus dorsi muscle. However, the IMF difference

between homozygote resistant and susceptible pigs is very small and is not interfering with breeding goals. No other important production trait was influenced by the resistant allele.

After inoculation of *E.coli* F4ac the amount of bacteria was raising until day 3 in susceptible piglets whereas in resistant piglets the number of growing colonies were almost back to normal at day 2 after infection. Resistant piglets had smaller ETEC colony counting numbers at any record day than susceptible ones.

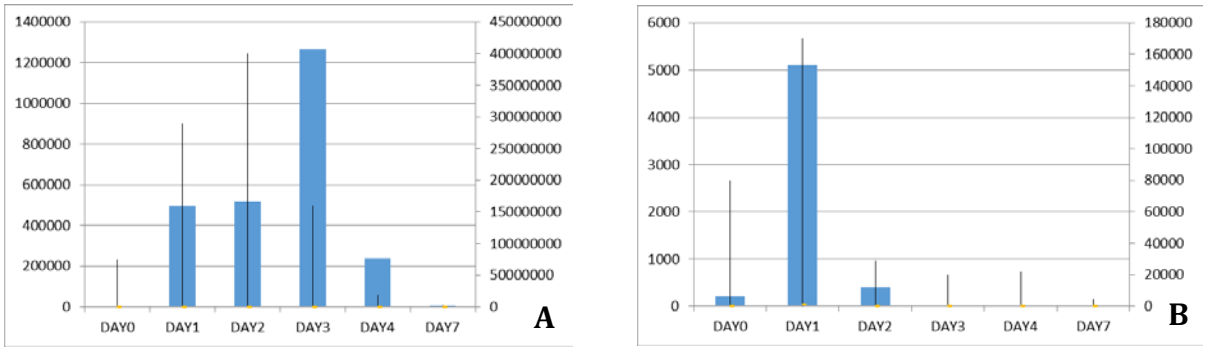

Figure 2: ETEC colony counting from the day before infection (day 0) to day 7 after infection. Figure 2A susceptible piglets, Figure 2B resistant piglets (note the different scale of the y-axis). Right Y-axis value means the maximum ETEC colony counting (the highest point of the black line) and left Y-axis value represents minimum, median, 75% point of the range and 25% of the range (blue box and yellow point).

Conclusion

Two flanking markers (*CHCF1* and *CHCF2*) with high linkage disequilibrium (LD) to the *F4bcR* were determined to effectively identify genetically F4ab/ac-resistant in Swiss Large White pigs. In Swiss Landrace, Piétrain and Duroc *CHCF1* and *CHCF3* were better markers than CHCF2. Although the molecular background of the *F4bcR* still needs to be resolved, breeding for *E.coli* F4-resistant pigs could be implemented into a breeding program using *CHCF1* and *CHCF3* as markers without a major impact on the production breeding values.

After inoculation of piglets genotyped as resistant *E. coli* F4ac bacteria proliferate to a much lesser extent than in piglets genotyped as susceptible. In addition, the time of spreading high numbers of pathogens was much shorter. We expect that the infection pressure on susceptible piglets decrease with an increasing amount of resistant piglets in the population.

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