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The possibility of positive selection for both F18$^+$ *Escherichia coli* and stress resistant pigs opens new perspectives for pig breeding

Annelies Coddens [a], Frank Verdonck [a], Martin Mulinge [a], Els Goyvaerts [d], Cora Miry [e], Bruno Goddeeris [b], Luc Duchateau [c], Eric Cox [a,*]

[a] *Laboratory of Veterinary Immunology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[b] *Department of Biosystems, Faculty of Bioscience Engineering, K.U. Leuven Kasteelpark Arenberg 30, B-3001 Heverlee, Belgium*
[c] *Department of Physiology and Biometrics, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium*
[d] *Dierengezondheidszorg Vlaanderen, Hagenbroeksesteenweg 167, 2500 Lier, Belgium*
[e] *Dierengezondheidszorg Vlaanderen, Industrielaan 29, 8820 Torhout, Belgium*

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Abstract

F18$^+$ *Escherichia coli* infections causing post-weaning diarrhoea and/or oedema disease are a major cause of economic losses in pig industry. To date, no preventive strategy can protect pigs from F18$^+$ *E. coli* infections. One of the most attractive approaches to eliminate F18$^+$ *E. coli* infections is the selection for pigs that are resistant to F18$^+$ *E. coli* infections. However, this strategy was not believed to be favourable because of reports of genetic association with the stress-susceptibility gene in the Swiss Landrace. To investigate this potential association more thoroughly, 131 randomly selected Belgian hybrid pigs were genotyped for both the F18$^+$ *E. coli* resistance alleles (*FUT1*^{A}) and the stress-susceptibility alleles (*RYR1*^{T}) and their association was investigated by determining the linkage disequilibrium. This linkage disequilibrium (LD = − 0.0149) is close to zero and does not differ significantly from 0 (likelihood ratio test $\chi^2_1 = 1.123$, $P = 0.29$), demonstrating no association between the *FUT1*^{A} and *RYR1*^{T} alleles. Furthermore, only a small fraction (4.6%) of the Belgian pigs was found to be resistant to F18$^+$ *E. coli* infections. Our results suggest that selection for F18$^+$ *E. coli* resistant pigs might be an attractive approach to prevent pigs from F18$^+$ *E. coli* infections, unlike to what has previously been postulated.

Keywords: F18$^+$ *Escherichia coli* (F107/86); Pig; *RYR1*; *FUT1*; Porcine stress syndrome

1. Introduction

Many pig farms suffer from severe economical losses due to outbreaks of post-weaning diarrhoea or

* Corresponding author. Tel.: +32 92647396; fax: +32 92647496.
E-mail address: Eric.Cox@UGent.be (E. Cox).

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oedema disease in young pigs. F18$^+$ *Escherichia coli* infections are a major cause of both diseases. A prerequisite for these infections is the adherence of F18$^+$ *E. coli* to specific F18 receptors (F18R) on the porcine gut epithelium, followed by colonization of the gut and excretion of entero- and/or verotoxins leading to diarrhoea and/or oedema disease, respectively.

The prevalence of F18$^+$ *E. coli* infections is estimated to be very high in Belgium (up to 96% of Flemish pig farms were seropositive) (Verdonck et al., 2003). Nevertheless, some pigs are found to be resistant to colonization by F18$^+$ *E. coli* due to lack of F18R expression. Extensive mating studies revealed that the F18R status of pigs is genetically determined with susceptibility dominating resistance (Bertschinger et al., 1993). The F18R locus has been genetically mapped to the halothane linkage group on porcine chromosome 6 and it was found that *FUT1*, encoding an α(1,2)fucosyltransferase, is a candidate gene for this locus (Meijerink et al., 1997; Meijerink et al., 2000). Sequencing of the *FUT1* gene revealed a polymorphism (G or A) at nucleotide 307. The F18$^+$ *E. coli* resistant pigs showed presence of the A nucleotide on both alleles ($FUT1^{A/A}$ genotype), whereas pigs susceptible to F18$^+$ *E. coli* had either the heterozygous $FUT1^{G/A}$ or the homozygous $FUT1^{G/G}$ genotype.

The HAL-locus, which also belongs to the halothane linkage group, determines whether pigs are susceptible for malignant hyperthermia, an inherited neuromuscular disorder that is triggered by inhalational anaesthetics (halothane), skeletal muscle relaxants (succinylcholine), as well as stress (Reik et al., 1983). Therefore, this disease is often referred to as the porcine stress syndrome (PSS). The underlying cause of this disease is the dysfunction of the Ca^{2+} release channel (ryanodine receptor, RYR) of the skeletal muscle sarcoplasmatic reticulum (O'Brien, 1986; Murayama et al., 2007), due to a mutation (C → T) at nucleotide 1843, resulting in substitution of Cys^{615} for Arg in the ryanodine receptor (Fujii et al., 1991).

In the present study, the prevalence of both the F18$^+$ *E. coli* resistance allele ($FUT1^A$) and the malignant hyperthermia susceptibility allele ($RYR1^T$) in the Belgian pig population is estimated. Furthermore, the association of the $FUT1^A$ alleles and the $RYR1^T$ alleles is investigated in order to address the possibility to select for both stress resistance and F18$^+$ *E. coli* resistance. Association of both alleles would have the negative side effect that an increase of the stress-susceptibility allele frequency would occur when selecting for F18$^+$ *E. coli* resistant pigs. Our results will enable us to formulate advices on the usefulness or potential drawbacks towards positive selection for F18$^+$ *E. coli* resistant pigs in the Belgian pig population.

2. Materials and methods

2.1. Survey sampling

Blood samples from pigs out of five Belgian provinces were randomly collected by veterinary practitioners and were sent to our laboratory by the regional veterinary investigation centres. Twenty-one percent of the examined pigs were sows and 79% were fattening pigs. The pigs had different ages and were randomly selected from both open and closed farms. The number of collected samples per province was accorded to the number of pig-rearing farms present in each province (West-Flanders $n = 67$, East-Flanders $n = 33$, Antwerp $n = 18$, Limburg $n = 10$, Flemish Brabant $n = 3$). A total of 131 pigs from 36 different farms with a maximum of five pigs per farm were successfully genotyped for both the F18$^+$ *E. coli* and stress-susceptibility genes. The selected pigs were not purebred, but hybrid in nature.

2.2. DNA extraction

DNA was extracted from whole blood using the following procedure. One hundred microliters blood was washed three times by adding 1 ml TE buffer (10 mM Tris–HCl + 1 mM EDTA; pH 7.5) followed by centrifugation at 3800 × *g* for 1 min. Next, 200 μl K-buffer (50 mM KCl + 20 mM Tris–HCl + 2.5 mM $MgCl_2$ + 0.5% Tween 20; pH 8.3) was added to the cell pellet, followed by 2 μl proteinase K (Boehringer Mannheim) and this mixture was incubated at 56 °C during 1 h. Inactivation of proteinase K was established by a 10-min incubation at 95 °C. After centrifugation (3800 × *g*, 1 min), the supernatant containing the genomic DNA was collected.