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# Unravelling the genetic components involved in the immune response of pigs vaccinated against influenza virus

Ricardo Zanella[a,b,1], Danielle Gava[a,1], Jane de Oliveira Peixoto[a], Rejane Schaefer[a], Janice Reis Ciacci-Zanella[a], Natalha Biondo[c], Marcos Vinicius Gualberto Barbosa da Silva[d], Maurício Egídio Cantão[a], Mônica Corrêa Ledur[a,*]

[a] Embrapa Swine and Poultry, Concórdia, SC, Brazil
[b] Present Address: University of Passo Fundo, Passo Fundo, RS, Brazil
[c] Santa Catarina State University, Lages, SC, Brazil
[d] Embrapa Dairy Cattle, Juíz de Fora, MG, Brazil

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ABSTRACT

A genome-wide association study for immune response to influenza vaccination in a crossbred swine population was conducted. Swine influenza is caused by influenza A virus (FLUAV) which is considered one of the most prevalent respiratory pathogens in swine worldwide. The main strategy used to control influenza in swine herds is through vaccination. However, the currently circulating FLUAV subtypes in swine are genetically and antigenically diverse and their interaction with the host genetics poses a challenge for the production of efficacious and cross-protective vaccines. In this study, 103 pigs vaccinated with an inactivated H1N1 pandemic virus were genotyped with the Illumina PorcineSNP60V2 BeadChip for the identification of genetic markers associated with immune response efficacy to influenza A virus vaccination. Immune response was measured based on the presence or absence of HA (hemagglutinin) and NP (nucleoprotein) antibodies induced by vaccination and detected in swine sera by the hemagglutination inhibition (HI) and ELISA assays, respectively. The ELISA test was also used as a measurement of antibody levels produced following the FLUAV vaccination. Associations were tested with $x^2$ test for a case and control data and using maximum likelihood method for the quantitative data, where a moderate association was considered if $p < 5 \times 10^{-5}$. When testing the association using the HI results, three markers with unknown location and three located on chromosomes *SSCX*, *SSC14* and *SSC18* were identified as associated with the immune response. Using the response to vaccination measured by ELISA as a qualitative and quantitative phenotype, four genomic regions were associated with immune response: one on *SSC12* and three on chromosomes *SSC1*, *SSC7*, and *SSC15*, respectively. Those regions harbor important functional candidate genes possibly involved with the degree of immune response to vaccination. These results show an important role of host genetics in the immune response to influenza vaccination. Genetic selection for pigs with better response to FLUAV vaccination might be an alternative to reduce the impact of influenza virus infection in the swine industry. However, these results should to be validated in additional populations before its use.



## 1. Introduction

In the livestock sector, most of the economic losses are caused by infectious pathogens (Miller et al., 2013). To mitigate those losses, several strategies have been proposed and implemented. Currently, vaccination is considered the most powerful tool to control and to diminish the disease burden, reducing the clinical signs and the transmission of the disease within and across herds (Kimman et al., 2007). However, the immune response to vaccination can vary among individuals, with animals not responding at all or with different levels of response (Poland et al., 2014; Rajao et al., 2014).

It has been proposed that the immune response to vaccination is inherited as a complex quantitative trait with variation resulting from environmental and genetic factors (Newport et al., 2004).

* Corresponding author at: Embrapa Swine and Poultry, Animal Health and Genetics Laboratory. BR 153, Km 110, Vila Tamanduá 89700-000, Concórdia, Santa Catarina, Brazil. Fax: +55 49 34410497.
*E-mail address:* monica.ledur@embrapa.br (M.C. Ledur).
[1] These authors contributed equally to this work.

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Some of the differences in the immune response to vaccination can be attributed to several factors other than the environmental ones, such as the vaccine itself, the vaccination procedure, and the ability of the host to respond to the immunization, which can be determined by sex, genetics, and age. Stressors and nutrition are likely to have a huge impact on the efficacy of the vaccine (Van Loveren et al., 2001). In addition, the induction of protective immunity generally requires the administration of multiple doses of vaccines, which can be labor intensive and expensive (Mitchell et al., 2013). Furthermore, it is known that not all animals are equally immunized in a commercial system. Therefore, better strategies to protect animals are needed (Meeusen et al., 2007).

Influenza A virus (FLUAV) is a zoonotic pathogen that causes an acute respiratory infection in humans and other animal species, and it is also considered a global health concern. The FLUAV infection in swine causes major economic impact in most pig-producing countries around the world due to the high morbidity observed in affected farms (Bennett, 2003; Bennett and Ijpelaar, 2005; Olsen et al., 2002). In Brazil, swine influenza virus is currently one of the most important pathogens of the porcine respiratory disease complex, and genetically diverse FLUAV subtypes (H1N1pdm, H1N2, and H3N2) are circulating in pig farms in most of the Brazilian states (Nelson et al., 2015; Rajao et al., 2013; Schaefer et al., 2011; Schaefer et al., 2015). Moreover, no commercial FLUAV vaccine was available in Brazil until July of 2014 (Schaefer et al., 2015).

Swine influenza vaccines are commonly used in commercial herds in several countries to control the clinical signs of the disease (Rajao et al., 2014). However, the effectiveness of the vaccines may vary in pig herds and multiple factors, as for example, the lack of cross-protective immunity due to the high genetic variability of FLUAVs, might hinder their success in controlling influenza outbreaks (Rajao et al., 2014). Several studies were conducted to evaluate the efficacy of different types of vaccines to FLUAV in a swine population showing different levels of immune response (Hoft et al., 2011; Masic et al., 2010; Sandbulte et al., 2014). Despite concerns regarding the prevention and transmission of swine influenza virus, no studies have investigated the interaction between the host genetics and the immune response to influenza vaccination. Therefore, understanding the genetic mechanisms involved with the immune response to FLUAV vaccination will contribute to the development of new methods to improve the vaccination efficacy, reducing and preventing the transmission of FLUAV within pig herds. Therefore, the objective of this study was to identify genetic markers associated with immune response to influenza vaccination in an experimental condition. To our knowledge, this is the first study evaluating the interaction of host genetics and the immune response to influenza vaccination in a swine population.

## 2. Material and methods

This study was conducted at Embrapa (Brazilian Agricultural Research Corporation) Swine and Poultry, Santa Catarina State, Brazil and had the approval of the Institutional Animal Care and Use Committee for all experimental protocols used (CEUA/CNPSA 004/2011).

### 2.1. Experimental population

One hundred and three piglets ($n = 103$, 55 females and 48 males) from three unrelated sires MS-115 (composite terminal sires developed by Embrapa Swine and Poultry) and 46 Landrace × Large White sows, with parity order ranging from one to six, were used in this study (Fig. 1). All sows were partially related to each other with an average identical-by-descent (IBD) of 0.18 (0.05–0.63).

The sows were kept in a group and moved to an individual farrowing crate ten days prior the farrowing. No cross fostering was performed with the experimental piglets to avoid interference of the maternal genetics and colostrum intake. After weaning, piglets were moved to a group-housing with seven piglets per pen. The grouped animals were kept together until the end of the experiment.

### 2.2. FLUAV vaccine preparation and vaccination of piglets

Influenza vaccine was prepared at Embrapa Swine and Poultry, using the virus strain A/swine/Brazil/107-3A/2010 (H1N1) (Genbank accession number KF683611–KF683618) isolated from piglets in southern Brazil in 2010, and genetically characterized as H1N1pdm virus (Schaefer et al., 2015). The virus antigen was propagated in Madin–Darby canine kidney (MDCK) cells at approximately 64HA units or $10^{4.38}$ 50% tissue culture infectious dose ($TCID_{50}$) per mL, as calculated by the Reed and Muench method (Reed and Muench, 1938). The virus inactivation was performed using a binary ethyleneimine (BEI) (Sigma–Aldrich). A commercial oil-in-water adjuvant (Emulsigen D, MVP Labs) at a v:v ratio of 4:1 virus to adjuvant was added to the inactivated virus, as described by Gauger and colleagues (Gauger et al., 2011).

Piglets were vaccinated with 1.5 mL of this vaccine via intramuscular route (neck) at approximately five weeks of age (34 days-old) and boosted at eight weeks of age (55 days-old) with the same dose. The age of the first vaccination was chosen based on preliminary data from our group that indicated the absence of maternal derived antibody (MDA) at this time (data not shown). The sows were not vaccinated against FLUAV since no commercial vaccine was available in Brazil during the experimental study (Schaefer et al., 2015).

### 2.3. Sample collection

The experimental study was run from January to June of 2014. Blood samples were collected through jugular puncture from piglets at 21, 34, 55, and 76 days-old and from sows at the weaning time for the detection of FLUAV antibodies (Fig. 1). Piglets body weight was measured at birth, weaning (21 days-old), and 21 days after the second vaccine dose (76 days-old). Lung tissue samples were collected from piglets during slaughter at 153 days-old and stored at −80 °C. In addition, ear tissue samples from 32 out of the 46 sows were also collected for genotyping. Fourteen sows were not collected because they were culled right after weaning.

### 2.4. Antibody detection assays

Antibodies produced against the hemagglutinin (HA) and the nucleoprotein (NP) of FLUAV were measured in swine sera by hemagglutination inhibition (HI) assay and ELISA, respectively.

The virus antigen in the HI assay was the same used for the vaccine preparation (A/swine/Brazil/107-3A/2010 (H1N1)), which was previously characterized in (Schaefer et al., 2015). For HI, sera were heat inactivated at 56 °C for 30 min, then treated with a 20% suspension of kaolin (Sigma–Aldrich), and adsorbed with 0.5% turkey red blood cells. The reciprocal of the highest serum dilution with a positive well was considered the HI titer. Antibody titers less than 10 were considered negative, titers between 10 and 20 were considered "suspect" and titers of 40 or greater were considered positive (Kitikoon et al., 2014).

The ELISA assay (Influenza A—Ab test; IDEXX® Laboratories Inc.) uses a monoclonal antibody generated against human influenza A virus subtype H1N1 and recognizes a highly conserved epitope of influenza A nucleoprotein (NP). Sample-to-negative (S/N) ratio was calculated according to the manufacturer instructions. Samples