

## EFFECT OF SELECTION FOR GROWTH RATE ON MACROSCOPIC LESIONS AFTER INTRADERMAL SKIN INFECTIONS WITH *STAPHYLOCOCCUS AUREUS*

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### ABSTRACT

Rabbits from paternal lines present some undesired characteristics such as poor reproductive performance or low stability on farms. Last fact suggests that selection could be getting worse the way selected animals respond to infectious challenges. To test this hypothesis, we performed an experiment that involved 73 2-month-old rabbits from a paternal line selected by average daily gain during the growing period at two levels of selection (VR18, generation 18; VR36, generation 36). These young rabbits were intradermally-inoculated in their backs with *S. aureus* of two rabbit strains of different virulence (Jwt, high virulence; and Jrot<sup>+</sup>, low virulence). Severity of lesions were evaluated by the presence and area of the erythema and nodules for 7 days. The presence of erythema and nodule was lower when inoculations were performed in animals from VR36 than when they were performed in animals from VR18 (-8.4 and -6.5 percentage points, respectively;  $P<0.05$ ). Nodules increased their size over time independently of the generation used for the infection. However, compared to VR18, animals from VR36 presented smaller nodules from day 3 after inoculation onwards ( $P<0.05$ ). Consequently, it seems that lesions caused by *S. aureus* in animals from VR36 were less severe than those caused in animals from VR18. These results are compatible with the fact that selection is not getting worse the way rabbits from paternal lines respond to infectious challenges.

**Key words:** Paternal line, R line, *Staphylococcal aureus*, Experimental infection, Pathology.

### INTRODUCTION

During the last 50 years, productivity of animals has increased considerably due to genetic selection. However, this selection has sometimes been accompanied by undesired side effects (Rauw et al., 1998). In this sense, rabbit females from paternal lines selected by growth rate seem to present poorer reproductive performance (Baselga, 2002) and stability on farms (Arnau-Bonachera et al., 2018) than females from maternal lines. Moreover, young rabbits from these lines are more sensitive to digestive disorders when antimicrobials are not used in the feedstuff (García-Quirós et al., 2014). However, with current information it is not clear whether these problems are the effect of genetic selection criteria or they are intrinsic to the breeds as a consequence of animals used at foundation. As semen from animals of the paternal lines is used in the final cross in commercial farms, a relevant effect of selection would mean that the susceptibility of young rabbits to pathogens is increasing with time. *S. aureus* is the causal agent of several pathological processes in growing rabbits and specially in rabbit females. In fact, it has been associated with mastitis of lactating does (Corpa et al., 2009), which is one of the main reasons of culling

females (Segura et al., 2007; Rosell and De la Fuente, 2009). Consequently, the study of *S. aureus* infections is relevant for rabbit farming. Moreover, it is possible to infect animals with this pathogen under controlled and standardized conditions (Viana et al., 2015) and there is literature about this kind of infections with different strains of the bacteria, different ages of animals, etc. Therefore, the aim of the present work was to evaluate the effect of selection by growth rate on the lesions observed after an experimental infection with *S. aureus*.

## MATERIALS AND METHODS

### Animals and experimental design

The experiment involved 73 2-month-old rabbits from a paternal line selected by average daily gain during the growing period over 38 generations (line R, Estany et al., 1992). To evaluate the effect of selection by average daily gain, we evaluated generation 18 (VR18) and 36 (VR36) using cryopreserved embryos. These embryos were transferred to females. After one generation without selection 32 rabbit females were selected to obtain the 73 young rabbits. Animals were housed under conventional environmental conditions with free access to water and to a commercial diet. To perform infections, animals were sedated with a combination of ketamine (Imalgene®, 100 mg/mL, Merial, Barcelona, Spain) and xylazine (Xilagesic, 200 mg/mL, Calier, Barcelona, Spain) and a 10x10 cm area of the dorsal-lumbar region was shaved and disinfected with chlorhexidine. Later, rabbits were intradermally-inoculated in their backs with 300 *S. aureus* colony forming units (CFU) of two rabbit strains of different virulence, Jwt (high virulence) and *Jrot*<sup>+</sup> (low virulence) (Viana et al., 2015), suspended in 0.1 mL of phosphate-buffered saline (PBS). Each rabbit was infected at four points (2 per strain). After inoculation, characteristics of the skin gross lesions (presence of erythema and/or nodules) were daily recorded for seven days. Erythema and abscess dimensions were measured with a caliper. The length (L) and width (W) values were used to calculate areas ( $A = \pi [L \times W]/2$ ).

### Statistical analysis.

The four evaluated traits in the present work were analysed using two different models. Both models included the effect of Generation (2 levels: VR18, VR36), Strain of *S. aureus* (2 levels: Jwt, *Jrot*<sup>+</sup>), day post-inoculation (7 levels: 1, 2, 3, 4, 5, 6, 7) and their interactions as fixed effects. For presence of erythema and nodule (dichotomic traits) a generalized linear model was used (proc GENMOD, SAS) after considering that the response variable followed a binomial distribution and by using a logistic transformation [ $\ln(\pi / (1-\pi))$ ] as a link function. For area of erythema and nodule a linear mixed model was performed (proc MIXED, SAS) including the permanent effects of animal [73 levels;  $N \sim (0, \sigma_p)$ ] and infection [292 levels;  $N \sim (0, \sigma_i)$ ], and residuals [2028 levels;  $N \sim (0, \sigma_e)$ ] as random effects. Assuming that measures of the same animal close in time are more correlated than far in time, residuals were considered to be correlated in a decreasing way as increasing the lag.

## RESULTS AND DISCUSSION

In Table 1 is presented the interaction between generation and strain for the four evaluated traits, as well as the contrasts between levels of the main effects. The presence of erythema and nodule was higher in infections inoculated with Jwt than with *Jrot*<sup>+</sup> (+12.0 and +51.2 percentage points, respectively;  $P < 0.001$ ). Moreover, lesions caused by Jwt were severer, with bigger erythema areas and nodules (+0.73 and +0.95 cm<sup>2</sup>, respectively;  $P < 0.001$ ). These results are in agreement with those reported previously (Viana et al., 2015) and denote the higher aggressiveness of the Jwt.

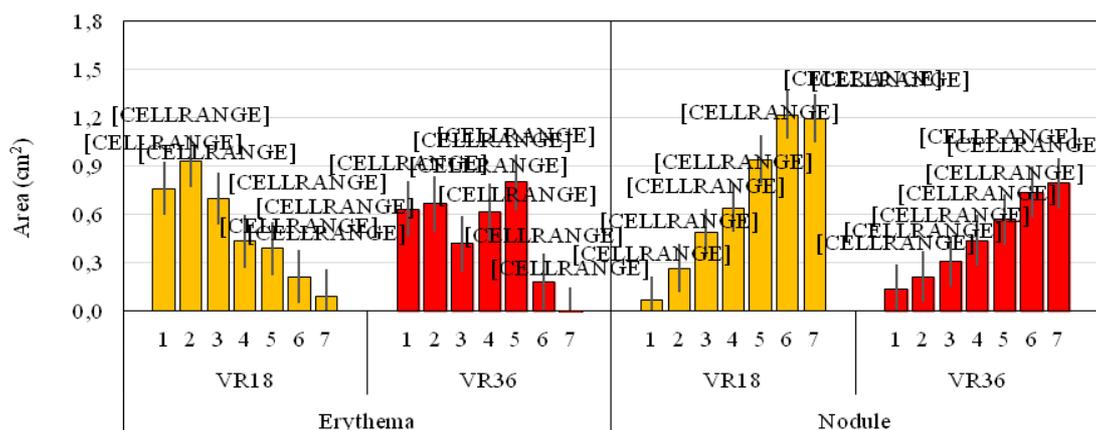
**Table 1:** Effect of generation, strain and their interaction on the presence and areas of erythema and nodules. Results are presented as least square means (standard errors into brackets).

	<i>Jrot</i> <sup>+</sup>		<i>Jwt</i>		Contrast	
	VR18	VR36	VR18	VR36	VR36-VR18	<i>Jwt</i> - <i>Jrot</i> <sup>+</sup>
Presence (%)						
Erythema	28.6 <sup>b</sup>	9.7 <sup>a</sup>	25.7 <sup>b</sup>	33.0 <sup>b</sup>	-8.4 <sup>**</sup>	12.0 <sup>***</sup>
Nodule	9.3 <sup>a</sup>	6.4 <sup>a</sup>	62.5 <sup>b</sup>	55.6 <sup>b</sup>	-6.5 <sup>*</sup>	51.2 <sup>***</sup>
Area (cm <sup>2</sup> )						
Erythema	0.24 <sub>(0.16)</sub> <sup>a</sup>	0.01 <sub>(0.16)</sub> <sup>a</sup>	0.77 <sub>(0.16)</sub> <sup>b</sup>	0.94 <sub>(0.16)</sub> <sup>b</sup>	-0.04 <sub>(0.13)</sub> <sup>*</sup>	0.73 <sub>(0.12)</sub> <sup>***</sup>
Nodule	0.13 <sub>(0.16)</sub> <sup>a</sup>	0.06 <sub>(0.16)</sub> <sup>a</sup>	1.24 <sub>(0.16)</sub> <sup>c</sup>	0.85 <sub>(0.16)</sub> <sup>b</sup>	-0.23 <sub>(0.11)</sub> <sup>*</sup>	0.95 <sub>(0.09)</sub> <sup>***</sup>

<sup>a, b, c</sup> Means in a row not sharing superscript differed significantly at  $P < 0.05$ .

Contrast significance: <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$ , <sup>\*\*\*</sup> $P < 0.001$ .

Respect to the effect of selection, the presence of erythema and nodule was lower when inoculations were performed in animals from VR36 than when they were performed in animals from VR18 (-8.4 and -6.5 percentage points, respectively;  $P < 0.05$ ). Moreover, the size of nodules was also smaller in animals from V36 (-0.23 cm<sup>2</sup>;  $P < 0.05$ ). However, these differences also depended on the strain of the inoculation. For the presence of erythema, the main difference between generations was observed for *Jrot*<sup>+</sup>, whereas no significant differences were observed for *Jwt*. On the other hand, the smaller size of the nodules in animals from VR36 was observed in inoculations with *Jwt*, but not in inoculations with *Jrot*<sup>+</sup>.



**Figure 1:** Evolution of the area of the erythema (left graph) and nodule (right graph) depending on the generation during the 7 days post-inoculation. Bars are the least square means and error lines are their standard errors. <sup>a-i</sup> Means not sharing superscript within the trait differed significantly at  $P < 0.05$ .

The evolution over time of the area of the erythema and nodule depending on the generation of selection is presented in Figure 1. The area of erythema presented a slightly different pattern between the animals of the two generations. In animals from VR18 the area of the erythema increased during the first 2 days after infections and decreased afterwards, however in animals from VR36 the area of erythema remained high until day 5. On the other hand, the area of the nodule reported more interesting information as nodules are related to the severity of the lesion (Viana et al., 2015). Nodules increased their size over time independently of the generation used for the infection. However, compared to VR18, animals from VR36 presented smaller nodules from day 3 after inoculation onwards ( $P < 0.05$ ). Consequently, it seems that lesions caused by *S. aureus* in animals from VR36 were less severe than those caused in animals from VR18. Results reported in the present work respect to the effect of selection are surprising as it is well documented that selection by productivity usually impairs negatively the immune response (Lochmiller

and Deerenberg, 2000; Rauw, 2012). This result could be related with natural selection or even it could be an indirect consequence of selecting healthy animals for the next generation (e.g. it is common discard animals with abscesses or females with sore hocks). Anyway, further research is required to find out the reasons of these discrepancies.

## CONCLUSIONS

The lower presence of erythema and nodule and the smaller nodules indicate that lesions caused by *S. aureus* in animals from VR36 were less severe than those caused in animals from VR18. These results are compatible with the fact that selection is not getting worse the way rabbits from paternal lines respond to intradermal infection with *S. aureus*.

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