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***Gunia M., Lantier F., Bed'hom B., Guitton E., Helies V. Helloin E., Herbert C.,  
Maupin M., Riou M., Robert R., Shrestha M., Garreau H.***

***PASTEURELLA MULTOCIDA EXPERIMENTAL INFECTION  
2): GENETIC PARAMETERS***

**Full text of the communication**

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## **PASTEURELLA MULTOCIDA EXPERIMENTAL INFECTION (2): GENETIC PARAMETERS**

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### **ABSTRACT**

In this study, we experimentally infected 953 crossbred rabbits from 6 sire lines with *Pasteurella multocida*, and diagnosed their response to Pasteurellosis. We recorded abscess and bacterial dissemination, growth before and after inoculation, and performed blood cell counts 14 days post inoculation. The heritabilities were low to moderate for all traits, except for the eosinophils percentage, which does not seem to be heritable. There were positive genetic correlations between the Resistance score and the red blood cell count, the hematocrit, hemoglobin and lymphocyte percentages. On the opposite, there were negative genetic correlations between the Resistance score and the platelets, the white blood cells, the neutrophil and monocytes percentages. Among the hematological traits, lymphocyte percentage could be a potential selection criterion to breed for Pasteurellosis resistance. Its heritability was relatively high ( $0.24 \pm 0.09$ ) compared to the other traits and its correlation with the Resistance score was also high (0.83). This is the first analyses of the genetic parameters of hematological traits in experimentally infected rabbits.

**Key words:** Pasteurellosis, resistance, hematology, blood cell counts, rabbit.

### **INTRODUCTION**

Pasteurellosis (*Pasteurella* infection) is one of the most common bacterial infection in rabbits, in commercial farms and laboratory facilities as well. Curative treatments using antibiotics are only partially efficient, with frequent relapses. Breeding rabbits for improved genetic resistance to Pasteurellosis would be a sustainable complementary approach. The objective of the study was to characterize the genetic variability of traits measured in rabbits experimentally infected with *Pasteurella multocida*. Such traits were related to the extent of bacterial and abscess dissemination, the growth of rabbits after inoculation, and the blood cell counts performed 14 days after inoculation. We also investigated the genetic correlation with the trait under selection in the commercial population.

### **MATERIALS AND METHODS**

#### **Animals**

The experimental trial is described in details in the companion paper (Gunia et al., 2020). Briefly, the experimental population includes 1001 crossbred rabbits: 48 controls and 953 inoculated. The 953 rabbits were inoculated with *Pasteurella multocida* and their response to the infection was studied for 14 days. Blood cell counts were performed on blood samples taken at the time of slaughter at day 14 post inoculation. The experimental crossbred animals were issued from sires of the 6 maternal lines of the 3 French rabbit breeders (Eurolap, Hycrole, Hypharm).

In addition, we studied production records in the selection populations. These selection populations include the purebred progenies of the sires used to produce the experimental population. The purebred progenies were raised in the facilities of the 3 breeding companies. Information on 11,971 purebred rabbits of 6 lines born in 2016 and 2017 was available.

### Traits

On the experimental crossbred population, the average daily weight gain from birth to weaning (ADG-BW) was measured before inoculation. After inoculation, we measured the following traits (Pasteurellosis resistance traits): Average daily weight gain during the first (ADG-PI1) or the second (ADG-PI2) week post inoculation, Abscess, Bacteria and Resistance scores. These scores (0 to 5) were given according to the extent of lesion and bacterial dissemination in the body. Resistance is a composite score taking into account survival, Abscess and Bacteria scores. The hematological traits were Red Blood Cells (RBC), White Blood Cells (WBC), Platelets (PLT) counts, the Hematocrit (HCT), Hemoglobin (HGB), and the percentages of Lymphocytes (LYM), Basophils (BAS), Neutrophils (NEU), Monocytes (MON), and Eosinophils (EOS). In this paper about Pasteurellosis resistance and hematological traits, we only took into account the traits measured on the inoculated rabbits. Records taken on the control group were not included in the analyses. On the purebred selection population, we considered the two main traits under selection across all the 6 lines, which are the number of kits born alive (NBA) and the weaning weight (WW).

### Statistical Analysis

The data were analyzed using Reml method with ASReml 3.0 linear mixed animal model. For more details see the paper of Shrestha *et al.* (2018). Heritabilities were estimated with a linear model using three trait analyses. These three traits analyses always included the two selection traits. Genetic and phenotypic correlations were estimated by including randomly two to four traits together.

## RESULTS AND DISCUSSION

The heritability of the hematological traits of the infected rabbits are presented in Table 1. The highest heritabilities was observed for the percentages of monocytes and lymphocytes. The heritability of the red blood cell traits (red blood cell count, hematocrit, hemoglobin) was not significantly different from zero. The heritability of the percentage of basophils was also not significantly different from zero and the percentage of eosinophils was not heritable. The platelet count was moderately heritable.

**Table 1:** Heritability of the hematological traits<sup>1</sup> measured on 574 experimentally infected crossbred rabbits

Trait	Heritability (SE)	Common litter effect (SE)
RBC	0.04 ± 0.06	
HCT	0.07 ± 0.07	
HGB	0.14 ± 0.08	
LYM	0.24 ± 0.09	
BAS	0.07 ± 0.06	
NEU	0.18 ± 0.08	
MON	0.30 ± 0.06	0.08 ± 0.06
EOS	0.00 ± 0.00	
WBC	0.17 ± 0.08	
PLT	0.19 ± 0.08	

<sup>1</sup>RBC=Red Blood Cells, HCT=Hematocrit, HGB=Hemoglobin, LYM=% Lymphocytes, BAS=% Basophils, NEU=% Neutrophils, MON=% Monocytes, EOS= % Eosinophils, WBC=White Blood Cells, PLT=Platelets.

The heritability of the disease resistance and production traits are presented in Table 2. They were in the same range as the heritability of the hematological traits.

**Table 2:** Heritability of disease resistance traits and production traits<sup>1</sup>

Type of traits	Trait	Population	Number of rabbits	Heritability (SE)	Environmental effect (SE) <sup>2</sup>
Pasteurellosis resistance	Abscess	Experimental	951	0.13 ± 0.07	0.07 ± 0.04
	Bacteria		951	0.08 ± 0.05	
	Resistance		953	0.16 ± 0.06	
	ADG-PI1	Experimental	902	0.29 ± 0.07	
	ADG-PI2		852	0.20 ± 0.06	
Production	ADG-BW	Experimental	980	0.11 ± 0.10	0.47 ± 0.05
	NBA	Selection	2,253	0.33 ± 0.06	0.10 ± 0.02
	WW	Selection	11,423	0.05 ± 0.02	0.23 ± 0.02

<sup>1</sup>ADG: Average daily weight gain; BW: from birth to weaning pre-inoculation; PI1: during first week post-inoculation; PI2: during second week post-inoculation; NBA: number of kits born alive; WW: weaning weight.

<sup>2</sup>Permanent environment effect for NBA, Common litter effect for Abscess, ADG-BW, and WW.

The correlations among hematological traits are presented in Table 3. We observed two groups of traits with positive correlations within the group and negative correlations with the traits of the other group. One group included the red blood cells traits (red blood cells, hematocrit and hemoglobin) and percentage of lymphocytes. The other group included white blood cells, platelets percentage of neutrophils and monocytes. No clear trend was observed for percentage of basophils.

**Table 3:** Genetic (above diagonal) and phenotypic (below diagonal) correlations<sup>1</sup> among hematological traits<sup>2</sup>

	RBC	HCT	HGB	LYM	BAS	NEU	MON	WBC	PLT
RBC		0.67	<b>0.87</b>	0.50	0.00	-0.02	-0.89	-0.85	-0.39
HCT	<b>0.98</b>		<b>0.98</b>	0.62	-0.63	-0.44	<b>-0.98</b>	<b>-0.84</b>	-0.80
HGB	<b>0.88</b>	<b>0.91</b>		0.51	-0.58	-0.38	<b>-0.91</b>	<b>-0.77</b>	-0.58
LYM	<b>0.18</b>	<b>0.26</b>	<b>0.24</b>		0.57	<b>-0.96</b>	<b>-0.69</b>	<b>-0.61</b>	<b>-0.60</b>
BAS	0	0.05	0.01	<b>0.43</b>		<b>-0.72</b>	0.39	-0.19	-0.63
NEU	-0.03	<b>-0.11</b>	-0.08	<b>-0.95</b>	<b>-0.49</b>		0.40	<b>0.54</b>	<b>0.67</b>
MON	<b>-0.25</b>	<b>-0.3</b>	<b>-0.42</b>	<b>-0.18</b>	<b>0.20</b>	-0.02		0.51	0.38
WBC	0.05	-0.03	0	<b>-0.67</b>	<b>-0.25</b>	<b>0.64</b>	0.06		<b>0.76</b>
PLT	0.05	0	-0.06	<b>-0.44</b>	<b>-0.20</b>	<b>0.44</b>	<b>0.17</b>	<b>0.49</b>	

<sup>1</sup>Bold: correlations significantly different from zero.

<sup>2</sup>RBC=Red Blood Cells, HCT=Hematocrit, HGB=Hemoglobin, LYM=% Lymphocytes, BAS=% Basophils, NEU=% Neutrophils, MON=% Monocytes, EOS= % Eosinophils, WBC=White Blood Cells, PLT=Platelets.

The Table 4 shows the genetic correlation between the hematological traits and the Pasteurellosis resistance or production traits. The correlations were moderate to high, however, only a limited number of correlations were significantly different from zero. The correlations were in accordance with the phenotypes observed in the first paper (Gunia *et al.*, 2020). Resistant rabbits tend to have higher red blood cells, hematocrit, hemoglobin and percentage of lymphocytes. They also had lower white blood cells, platelets, percentage of neutrophils and monocytes. Red blood cells, hematocrit, hemoglobin and percentage of lymphocytes had a positive genetic correlation with Resistance, average daily gain pre and post inoculation, and lower with Abscess and Bacteria. White blood cells, neutrophils, monocytes, and platelets showed the opposite trend. The genetic correlations between the hematological traits and the two selection traits were not significantly different from zero, probably due to a lack of power of our study. They tend to be mostly positive with weaning weight (except for white blood cells, hematocrit, and platelets) and mostly negative with the number of kits born alive (except for neutrophils).

Percentages of Lymphocytes and Neutrophils were the two traits with the highest correlations with the other disease resistance traits. They seem to be the strongest indicators of resistance or sensitivity to Pasteurellosis. Neutrophils are involved in abscess formation. The high response for neutrophils could be explained by the choice of a virulent *Pasteurella multocida* strain that causes abscesses in rabbits (Helloin *et al.*, 2015). Lymphocytes are a key component of the adaptive immune response. Due to its higher

heritability and genetic correlation with Resistance, the lymphocyte percentage could be an interesting selection criterion to breed rabbits for improved Pasteurellosis resistance. Further studies are needed to analyze the response in the lymphocyte sub-populations.

The genetic correlations were stronger between the hematological traits and Abscess or the Average Daily Gain during the 2<sup>nd</sup> week post inoculation than with the other traits. This could be explained by the fact that these traits directly reflect the efficiency of the immune response, directly for Abscess and indirectly for ADG2.

**Table 4:** Genetic correlations between hematological traits<sup>1</sup> and Pasteurellosis resistance traits<sup>2</sup> or production traits<sup>3</sup>

	ABSCESS	BACTERIA	RESISTANCE	ADG1	ADG2	ADGBW	WW	NBA
RBC	-0.42	0.21	0.33	0.43	<b>0.74</b>	0.73	0.02	-0.89
HCT	<b>-0.62</b>	-0.28	0.54	<b>0.50</b>	<b>0.79</b>	0.87	-0.06	-0.49
HGB	<b>-0.55</b>	-0.32	0.47	0.34	<b>0.72</b>	0.99	0.18	-0.40
LYM	<b>-0.95</b>	-0.21	<b>0.83</b>	<b>0.44</b>	<b>0.84</b>	0.24	0.14	-0.39
BAS	-0.43	-0.38	0.27	0.39	0.35	-0.60	0.03	-0.12
NEU	<b>0.89</b>	<b>0.91</b>	<b>-0.76</b>	-0.71	<b>-0.73</b>	-0.87	<b>0.36</b>	0.42
MON	<b>0.65</b>	<b>0.57</b>	-0.47	-0.29	<b>-0.54</b>	-0.75	0.26	-0.45
WBC	0.50	0.59	-0.40	-0.29	<b>-0.84</b>	-0.90	-0.32	-0.07
PLT	<b>0.61</b>	0.52	-0.43	-0.14	<b>-0.96</b>	-0.25	-0.13	-0.12

<sup>1</sup>Hematological traits: RBC=Red Blood Cells, HCT=Hematocrit, HGB=Hemoglobin, LYM=Lymphocytes, BAS=Basophils, NEU=Neutrophils, MON=Monocytes, WBC=White Blood Cells, PLT=Platelets

<sup>2</sup>Pasteurellosis resistance traits: abscess, bacteria and resistance score, ADG1 and ADG2=Average Daily Gain the 1st and 2nd week post inoculation

<sup>3</sup>Production traits: ADGBW=Average Daily Gain before weaning, WW=Weaning weight and NBA=Number of kits Born Alive  
Bold: correlations significantly different from zero.

## CONCLUSIONS

There was substantial genetic variation in the response to *Pasteurella multocida* infection. The heritability of traits related to white blood cells measured post infection were all significantly different from zero, with the exception of the percentage of eosinophils. On the opposite, heritability of the traits related to red blood cells were not significantly different from zero. The lymphocytes and neutrophils percentage were highly genetically correlated with the resistance or sensitivity to Pasteurellosis.

## ACKNOWLEDGEMENTS

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## ➤ *Pasteurella Multocida experimental infection (2):*

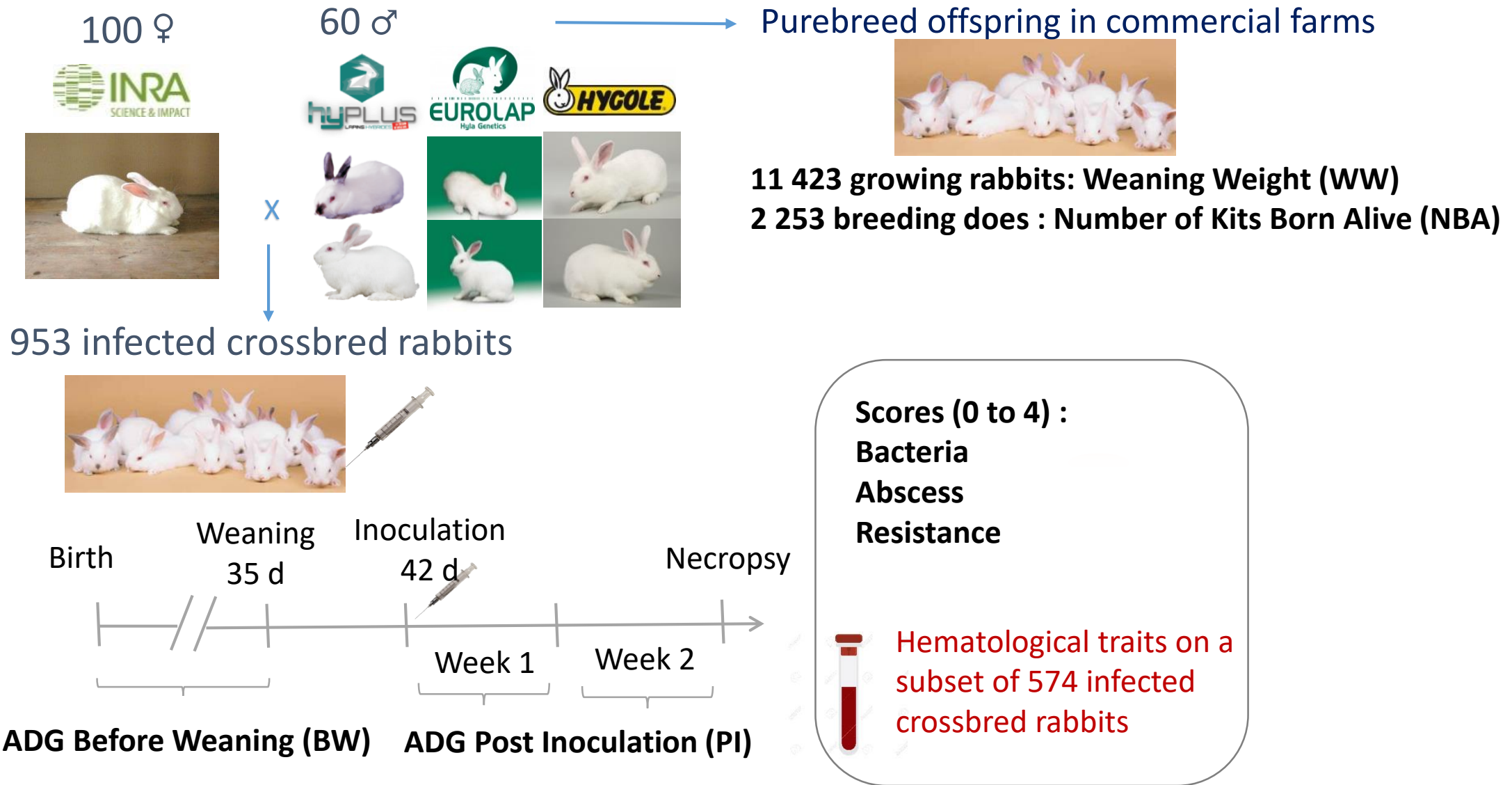
*Genetic parameters of hematological traits in response to a Pasteurella Multocida experimental infection in rabbits*

Gunia M., Lantier F., Bed'hom B., Guitton E., Helies V., Helloin E., Herbert C., Maupin M., Riou M., Robert R., Garreau H.

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# Experimental design



# Hematological traits

## Red blood cells

RBC	Red blood cells	$10^6/\text{mm}^3$	
HCT	Hematocrit	%	% RBC in the blood
HGB	Hemoglobin (protein carrying oxygen in the RBC)	g/dl	Level of hemoglobin in the blood

## White blood cells

LYM	Lymphocytes	$10^3/\text{mm}^3$	Acquired immunity
BAS	Basophils	$10^3/\text{mm}^3$	Innate immunity
NEU	Neutrophils	$10^3/\text{mm}^3$	
MON	Monocytes	$10^3/\text{mm}^3$	
WBC	White blood cells	$10^3/\text{mm}^3$	

## Platelets

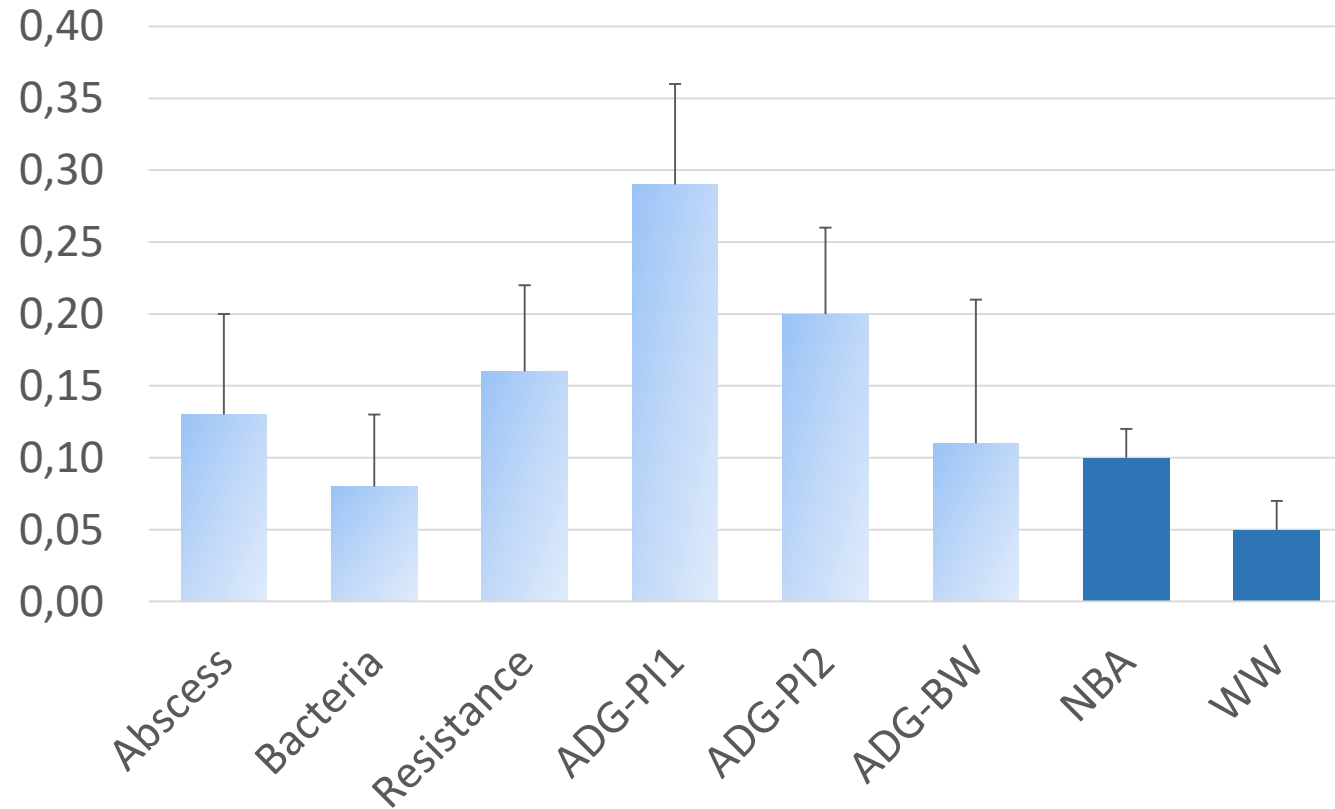
PLT	Platelets	$10^3/\text{mm}^3$
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# Heritability

## Disease resistance and production traits

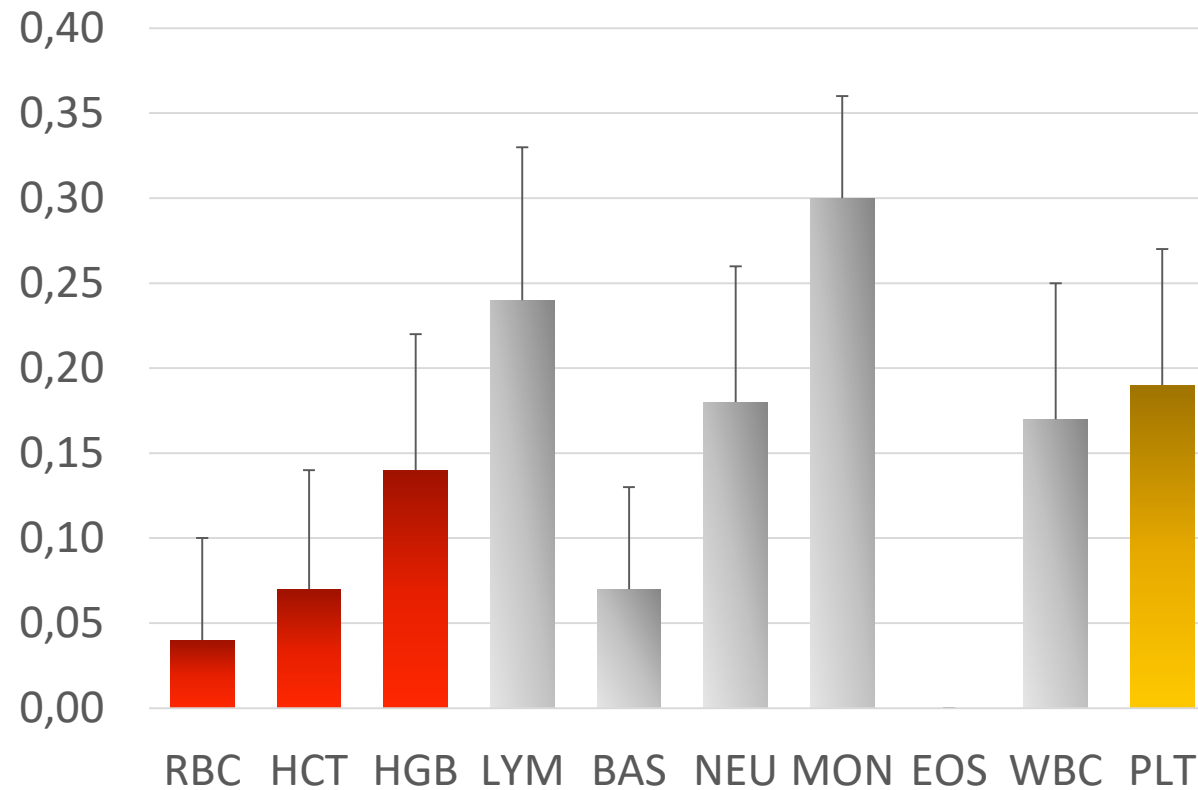


Low to moderate heritabilities



# Heritability

## Hematological traits



## Genetic correlations between hematological traits and Pasteurellosis resistance traits or production traits

	ABSCESS	BACTERIA	RESISTANCE	ADG1	ADG2	ADG-BW	WW	NBA
<b>RBC</b>	-0.42	0.21	0.33	0.43	<b>0.74</b>	0.73	0.02	-0.89
<b>HCT</b>	<b>-0.62</b>	-0.28	0.54	<b>0.50</b>	<b>0.79</b>	0.87	-0.06	-0.49
<b>HGB</b>	<b>-0.55</b>	-0.32	0.47	0.34	<b>0.72</b>	0.99	0.18	-0.40
<b>LYM</b>	<b>-0.95</b>	-0.21	<b>0.83</b>	<b>0.44</b>	<b>0.84</b>	0.24	0.14	-0.39
BAS	-0.43	-0.38	0.27	0.39	0.35	-0.60	0.03	-0.12
NEU	<b>0.89</b>	<b>0.91</b>	<b>-0.76</b>	-0.71	<b>-0.73</b>	-0.87	0.36	0.42
MON	<b>0.65</b>	<b>0.57</b>	-0.47	-0.29	<b>-0.54</b>	-0.75	0.26	-0.45
WBC	0.50	0.59	-0.40	-0.29	<b>-0.84</b>	-0.90	-0.32	-0.07
<b>PLT</b>	<b>0.61</b>	0.52	-0.43	-0.14	<b>-0.96</b>	-0.25	-0.13	-0.12

Positive genetic correlation between:  
 Red blood cells, hematocrit, hemoglobin, lymphocytes  
 and  
 Resistance, ADG pre and post inoculation



## Genetic correlations between hematological traits and Pasteurellosis resistance traits or production traits

	ABSCESS	BACTERIA	RESISTANCE	ADG1	ADG2	ADG-BW	WW	NBA
<b>RBC</b>	-0.42	0.21	0.33	0.43	<b>0.74</b>	0.73	0.02	-0.89
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WBC	0.50	0.59	-0.40	-0.29	<b>-0.84</b>	-0.90	-0.32	-0.07
<b>PLT</b>	<b>0.61</b>	0.52	-0.43	-0.14	<b>-0.96</b>	-0.25	-0.13	-0.12

Negative genetic correlation between:  
 White blood cells, neutrophils, monocytes, and platelets  
 and  
 Resistance, ADG pre and post inoculation



## Genetic correlations between hematological traits and Pasteurellosis resistance traits or production traits

	ABSCESS	BACTERIA	RESISTANCE	ADG1	ADG2	ADG-BW	WW	NBA
RBC	-0.42	0.21	0.33	0.43	<b>0.74</b>	0.73	0.02	-0.89
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PLT	<b>0.61</b>	0.52	-0.43	-0.14	<b>-0.96</b>	-0.25	-0.13	-0.12

Genetic correlations between the hematological traits and selection traits not significantly different from zero



## Genetic correlations between hematological traits and Pasteurellosis resistance traits or production traits

	ABSCESS	BACTERIA	RESISTANCE	ADG1	ADG2	ADG-BW	WW	NBA
RBC	-0.42	0.21	0.33	0.43	<b>0.74</b>	0.73	0.02	-0.89
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PLT	<b>0.61</b>	0.52	-0.43	-0.14	<b>-0.96</b>	-0.25	-0.13	-0.12

Highest correlations with disease resistance traits:

- Neutrophils : involved in abscess formation
  - Lymphocytes : key component of the adaptive immune response, more heritable
- ➡ interesting selection criterion for improved Pasteurellosis resistance

## ➤ Conclusion

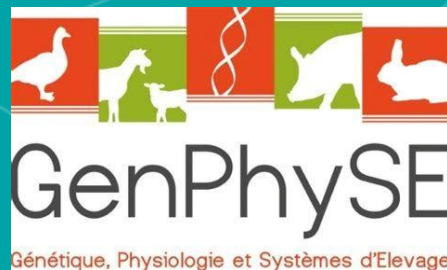
- Low to moderate heritability for hematological traits under *Pasteurella multocida* infection
- No significant correlations between hematological traits and selection traits
- Higher Red blood cells, hemoglobin and hematocrit associated with a higher resistance
- Higher Lymphocytes associated with a higher resistance ➡ interesting selection criterion for improved Pasteurellosis resistance





➤ Thank you for your attention

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Maupin M., Riou M., Robert R., Garreau H.





# Mean and standard deviation of hematological traits

Hematological parameter	Unit	Control (n=28)	Inoculated rabbits (n=574)
Red Blood Cells	$10^6/\text{mm}^3$	$5.46 \pm 0.76$	$5.00 \pm 0.71$
Hematocrit	%	$35.30 \pm 5.12$	$30.61 \pm 4.83$
Hemoglobin	g/dl	$11.89 \pm 1.61$	$10.52 \pm 1.41$
Lymphocytes	%	$61.34 \pm 7.20$	$32.46 \pm 12.82$
Basophils	%	$0.47 \pm 0.14$	$0.36 \pm 0.14$
Neutrophils	%	$26.56 \pm 6.29$	$52.00 \pm 12.80$
Monocytes	%	$7.19 \pm 1.60$	$9.51 \pm 2.47$
Eosinophils	%	$1,48 \pm 0,66$	$3.23 \pm 3.10$
White Blood Cells	$10^3/\text{mm}^3$	$6.53 \pm 2.52$	$12.66 \pm 6.78$
Platelets	$10^3/\text{mm}^3$	$201.02 \pm 106.33$	$333.31 \pm 199.98$

