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## PATHOLOGY & HYGIENE

***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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# *PASTEURELLA MULTOCIDA* EXPERIMENTAL INFECTION 1): RESISTANCE AND HEMATOLOGICAL RESPONSE

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## ABSTRACT

Pasteurellosis is the first cause of female mortality in rabbit farms. During the RELAPA project (Genomics for the Genetic Resistance of Rabbits to Pasteurellosis), 953 rabbits were inoculated at 6 weeks of age with a pyogenic strain of *Pasteurella multocida* (*Pm*) and were monitored during 14 days. Disease response was very variable among animals, with 7% of resistant animals and 11% of highly susceptible rabbits. Blood cell counts were performed at day 14 after inoculation on 574 inoculated and 28 control rabbits. Significant differences in white blood cell, red blood cell, and platelet counts were observed according to the disease resistance score. Susceptible rabbits have a lower red blood cell count, probably due to the hemolytic and hemorrhagic activity of *Pm*. They also have a higher percentage of monocytes, neutrophils and eosinophils (mainly involved in the innate immune system and inflammatory responses) and, conversely, a lower percentage of lymphocytes (mainly involved in the adaptive immune response) compared to highly resistant and control rabbits. They do not seem able to mount an effective immune response to control the infection.

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

## INTRODUCTION

Pasteurellosis is one of the most common bacterial infection in commercial rabbit farms. Pneumonia, caused mainly by *Pasteurella multocida (Pm*), are a major cause (37.8%) of female mortality on farms (Lopez et al. 2013). Genetic selection for resistance to Pasteurellosis is therefore an important goal. Following the collection and characterization of *Pm* strains (Helloin et al., 2013), the RELAPA project (Genomics for the Genetic Resistance of Rabbits to Pasteurellosis) aims at identifying genomic regions associated with the resistance to Pasteurellosis. This study presents the results of the blood cells count performed after the experimental inoculation of rabbits with a strain of *Pm*.

## MATERIALS AND METHODS

All experiments were conducted in accordance with the guidelines of the directive 2010/63/EU of the European Parliament and of the Council, in the facilities of the Plate-Forme d'Infectiologie Expérimentale: PFIE, UE-1277, INRA Centre Val de Loire, Nouzilly, France. All experimental procedures were approved by the Loire Valley ethical review board (CEEA VdL, committee number 19, N° APAFiS#3866).

**Animals**

A total of 1030 rabbits were produced Pôle d'expérimentation cunicole toulousain (PECTOUL) by mating 111 dams of the INRA 1777 maternal line and 65 sires of the 6 maternal lines of the 3 French breeders of rabbits (Eurolap, Hycole, Hypharm). Rabbits were produced in 5 batches. They were weaned at 35 days and transported the day after weaning to the Plateforme d'Infectiologie Expérimentale (PFIE, https://doi.org/10.15454/1.5572352821559333e12). A total of 953 inoculated rabbits and 48 controls were kept for this study; 27 rabbits of the inoculation group and 2 rabbits of the control group were excluded from the study due to early mortality not associated with Pasteurellosis. These animals had diarrhea or Epizootic Rabbit Enteropathy (ERE) symptoms without any *Pm* detected.

**Experimental design and measured traits**

The animals were housed in cages of five, in two rooms, with a balanced distribution of sexes and paternal and maternal origins. After one week of adaptation, the rabbits were infected at the age of 42 days (D0), by subcutaneous injection between the shoulder blades of a standardized dose of 8,000 bacteria / 0.1 ml saline of the pyrogenic *Pm* strain CIRMBP-0884 from a stock kept frozen at -80°C and checked for its concentration at each inoculation batch (Helloin et al., 2015). The health status of the rabbits was monitored daily for 14 days post-inoculation. Critically ill rabbits were euthanized for welfare reasons. The animals were weighed on days 1, 7 and 14. Their body temperature was measured daily from D-1 to D10. With the exception of animals that died or were euthanized during the study, all the rabbits were euthanized at day 14 day after inoculation (56 days of age) and their bodies were examined for signs of pasteurellosis. . At the post mortem examination, the presence and distribution of abscesses were recorded.

**Samples**

Spleen, lungs, liver and abscess samples were collected for bacteriological analyzes. At the time of slaughter at D14, blood samples were collected during the bleeding after electroconarcosis on a subset of 602 rabbits (574 inoculated rabbits and 28 controls) of batches 2, 3, 4 and 5. Blood cell counts were carried out with the MS9-5 Hematology Counter® (Melet Schloesing Laboratories, France).

**Traits and statistical analyzes**

Rabbits were scored on the basis of post-mortem examination results for the extent and severity of the abscesses. Another score was given based on the extent of bacterial dissemination in organs (Gunia et al., 2017). These abscess and bacteriological scores (from 0 to 4) were then combined to create a resistance score (Figure 1). The hematological parameters studied are described in Table 1. White blood cells or leucocytes include lymphocytes, monocytes, neutrophils, eosinophils and basophils. These cells are involved in the inflammation or in the innate immune system. Lymphocytes are a key component of the adaptive immune system, which is more pathogen- specific. Red blood cells transport oxygen and platelets coagulate blood. The hematocrit is the volume percentage of red blood cells in blood. Hemoglobin is a protein found in red blood that carries oxygen. The average values of the hematological parameters were estimated based on the 602 individuals. The relation between hematological characters and resistance score were studied on 601 individuals: 74 rabbits with a score of 1, 425 with a score of 2, 17 with a score of 3, 57 with a score of 4 and 28 controls. The only individual with a resistance score of 0 was excluded from these analyzes. The relationships between the resistance score on the one hand and the hematological parameters on the other hand were analyzed with a linear model (Proc glm of the SAS software).

## RESULTS AND DISCUSSION

The proportion of rabbits according to their resistance score is presented in Figure 1 and the average values of hematological parameters in the control and inoculated groups are presented in Table 1.
Figure 2 shows the levels of red blood cells, hematocrit, hemoglobin and platelets. The resistant, highly resistant and control rabbits have a significantly higher red blood cell count compared to the susceptible and intermediate animals. Logically, the hematocrit and the hemoglobin concentration

follow the same trend. Platelet counts follow the opposite trend, with the highly resistant animals and controls having a lower level than susceptible animals. This can be explained by the hemolytic and hemorrhagic activities of *Pm* toxins, also observed at post-mortem examination by the petechiae (infiltrations of blood) observed on the internal organs of susceptible animals. As a result, these animals mobilize more platelets to limit blood loss.

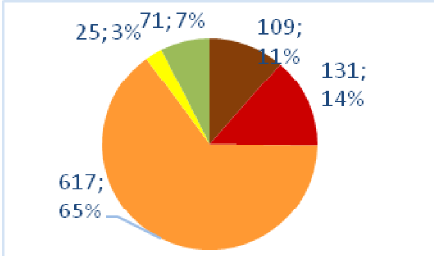
| Score | Description | Proportion and number of rabbits per score |
|---|---|---|
| 0 | Abscesses or growth of *Pm* in the culture from the tissue samples, dead or euthanized during the experiment |  |
| 1 | Abscess observed inside the organs and cavities, growth of *Pm* in the culture from the tissue samples, alive at day 14 | |
| 2 | Subcutaneous abscesses, alive at day 14 | |
| 3 | Abscess at the inoculation site only, alive at day 14 | |
| 4 | No abscess, no *Pm* growth in the culture, alive at day 14 | |



**Figure 1:** Pasteurellosis resistance score.

**Table 1**: Means and standard deviation of the hematological parameters for the inoculated rabbits (total and per resistance score) and for the control group

| Hematological parameter | Unit | Inoculated rabbits | Resistance scores of the inoculated rabbits | | | | Control (n=28) |
|---|---|---|---|---|---|---|---|
| | | Total (n=574) | 1 (n=74) | 2 (n=425) | 3 (n=17) | 4 (n=57) | |
| Red Blood Cells | $10^6$/mm$^3$ | 5.00 ± 0.71 | 4.72 ± 0.86 | 4.97 ± 0.67 | 5.46 ± 0.31 | 5.44 ± 0.67 | 5.46 ± 0.76 |
| Hematocrit | % | 30.61 ± 4.83 | 28.56 ± 5.44 | 30.14 ± 4.33 | 34.06 ± 3.27 | 35.69 ± 4.29 | 35.30 ± 5.12 |
| Hemoglobin | g/dl | 10.52 ± 1.41 | 10.13 ± 1.58 | 10.32 ± 1.25 | 11.66 ± 1.14 | 12.13 ± 1.22 | 11.89 ± 1.61 |
| Lymphocytes | % | 32.46 ± 12.82 | 28.11 ± 9.87 | 29.31 ± 9.03 | 45.36 ± 13.48 | 57.97 ± 8.33 | 61.34 ± 7.20 |
| Basophils | % | 0.36 ± 0.14 | 0.34 ± 0.13 | 0.34 ± 0.12 | 0.42 ± 0.13 | 0.49 ± 0.16 | 0.47 ± 0.14 |
| Neutrophils | % | 52.00 ± 12.80 | 54.79 ± 11.84 | 54.86 ± 10.17 | 42.09 ± 12.60 | 29.84 ± 7.72 | 26.56 ± 6.29 |
| Monocytes | % | 9.51 ± 2.47 | 9.93 ± 2.59 | 9.74 ± 2.47 | 8.22 ± 1.64 | 7.66 ± 1.31 | 7.19 ± 1.60 |
| Eosinophils | % | 3.23 ± 3.10 | 4.39 ± 4.26 | 3.36 ± 2.97 | 1.44 ± 1.19 | 1.33 ± 0.68 | 1,48 ± 0,66 |
| White Blood Cells | $10^3$/mm$^3$ | 12.66 ± 6.78 | 12.99 ± 6.79 | 13.46 ± 6.84 | 9.68 ± 5.64 | 6.96 ± 2.16 | 6.53 ± 2.52 |
| Platelets | $10^3$/mm$^3$ | 333.31 ± 199.98 | 354.22 ± 190.24 | 347.65 ± 207.00 | 273.38 ± 132.89 | 210.58 ± 107.09 | 201.02 ± 106.33 |

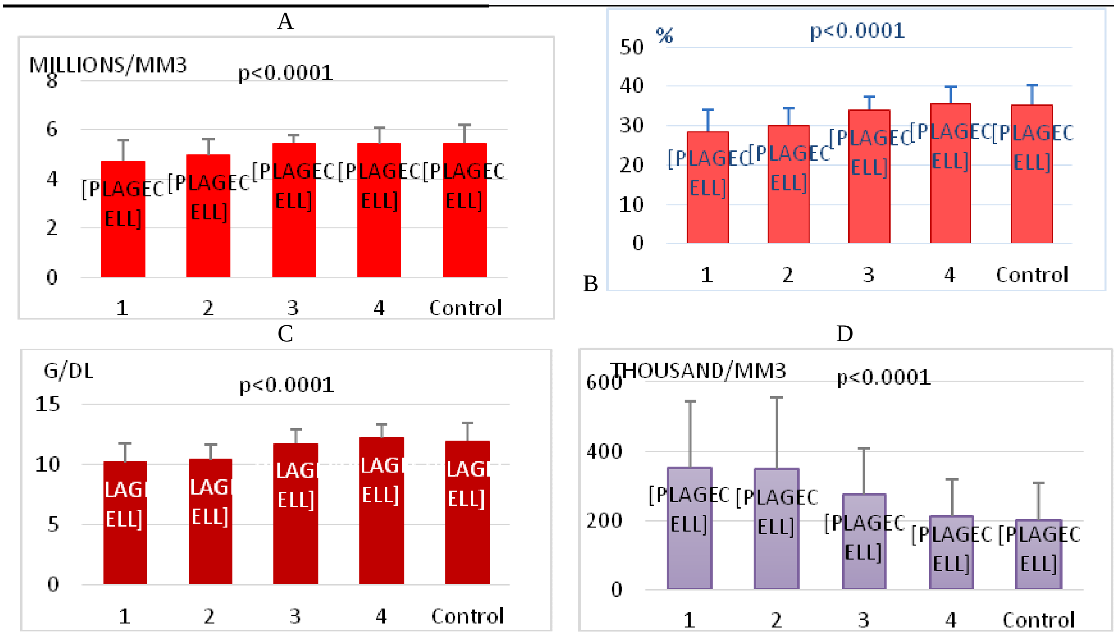



**Figure 2: A.** Red blood cells count **B.** Hematocrit **C.** Hemoglobin and **D.** Platelets according to the resistance score from 0 (highly susceptible) to 4 (highly resistant)

Figure 3 shows the number of leucocytes and the proportion of the different subsets of leucocytes. The highly resistant animals and the controls have a lower number of leucocytes than the susceptible and very susceptible animals, the resistant animals (score of 3) being intermediate. The proportions of each type of leukocytes and their numbers (not shown here) are significantly different between rabbit groups based on the resistance score. Susceptible animals have more monocytes, neutrophils, eosinophils (involved in the innate immune system). Monocytes and neutrophils are involved in phagocytosis eosinophils are involved in defense against parasites and some bacterial infections. Neutrophils are contributing to the formation of abscesses in response to local bacterial proliferation. Susceptible animals have fewer lymphocytes (implicated in the adaptive immune system) and basophils (involved in inflammation). It can be deduced that 14 days after inoculation, the most resistant animals would have mounted an effective immune response (innate and perhaps adaptive), which would not be the case of the susceptible rabbits.

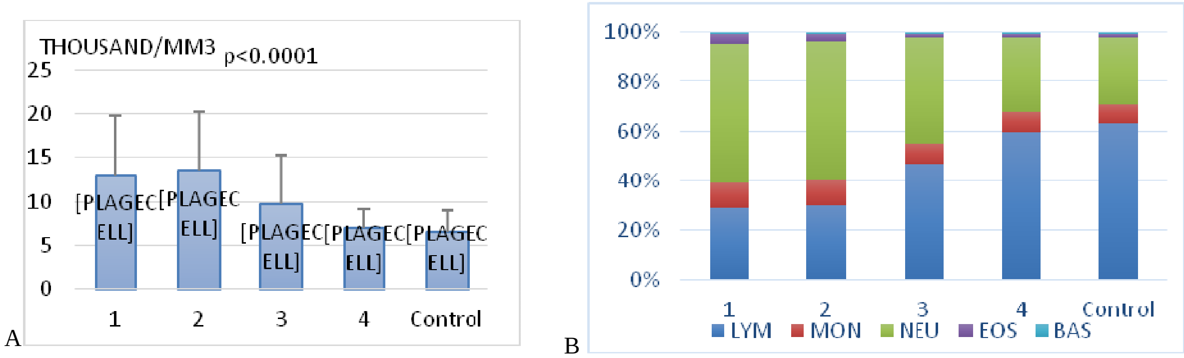



**Figure 3: A.** White blood cells and **B.** Percentage of white blood cells type per resistance score from 0 (highly susceptible) to 4 (highly resistant). LYM=Lymphocytes, MON=Monocytes, NEU=Neutrophils, EOS=Eosinophils, BAS=Basophils.

## CONCLUSIONS

This first step of the RELAPA project shows significantly different hematological profiles between resistant and susceptible animals. Susceptible rabbits have a lower red blood cell count, probably due to the hemolytic and hemorrhagic activity of *Pm*. They also have a higher percentage of monocytes, neutrophils and eosinophils (involved in the innate immune system and inflammatory responses) and a lower percentage of lymphocytes (involved in the adaptive immune response) compared to highly resistant and control rabbits. They do not seem able to mount an effective immune response to control the infection. This results show a large variability between rabbits in the response to Pasteurellosis infection, which may have a genetic origin.

## ACKNOWLEDGEMENTS

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# *Pasteurella Multocida experimental infection (1):*

## Resistance and hematological response

**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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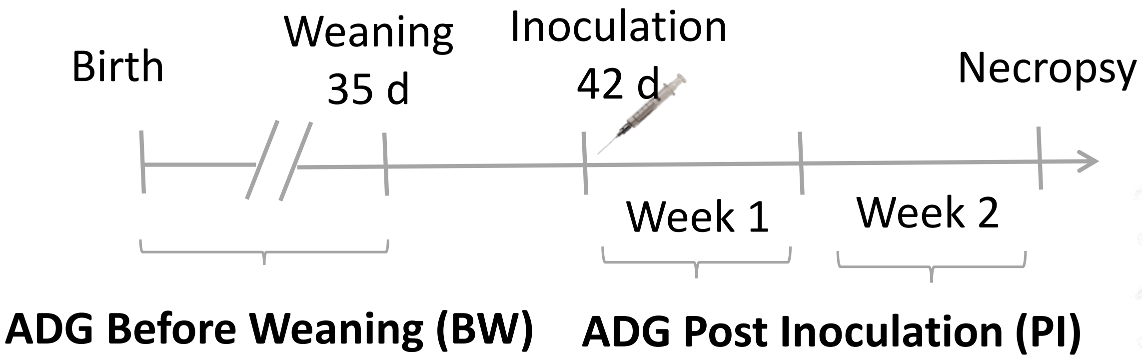
# Experimental design

100 ♀

X

60 ♂

953 infected and 48 control crossbred rabbits

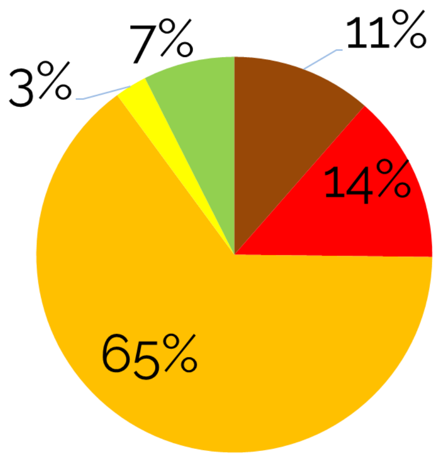



**Scores (0 to 4) :**
**Bacteria**
**Abscess**
**Resistance**

Hematological traits on a subset of 574 infected and 28 control crossbred rabbits

# Resistance scores

| | |
|---|---|
| 0 | Abscesses or Pasteurella in the tissues, dead or euthanized during the experiment |
| 1 | Abscess inside the organs and cavities, alive at day 14 |
| 2 | Subcutaneous abscesses, alive at day 14 |
| 3 | Abscess at the inoculation site only, alive at day 14 |
| 4 | No abscess, no Pm growth in the culture, alive at day 14 |



# Hematological traits

Red blood cells

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

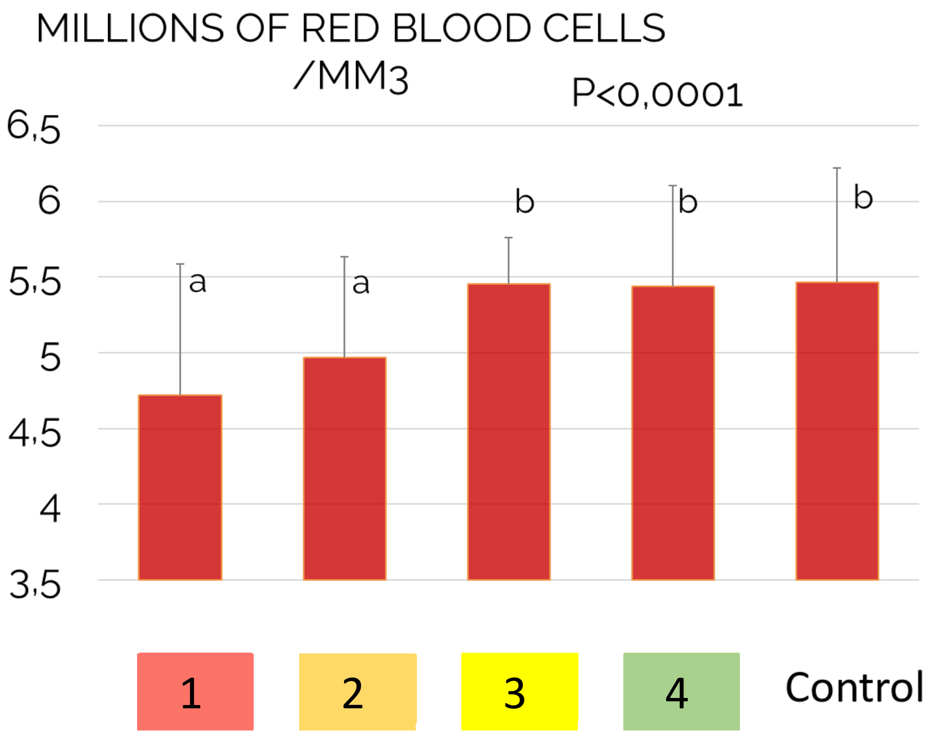
White blood cells

| | | | |
|---|---|---|---|
| LYM | Lymphocytes | $10^3/mm^3$ | Acquired immunity |
| BAS | Basophils | $10^3/mm^3$ | Innate immunity |
| NEU | Neutrophils | $10^3/mm^3$ | |
| MON | Monocytes | $10^3/mm^3$ | |
| WBC | White blood cells | $10^3/mm^3$ | |

Platelets

| | | |
|---|---|---|
| PLT | Platelets | $10^3/mm^3$ |

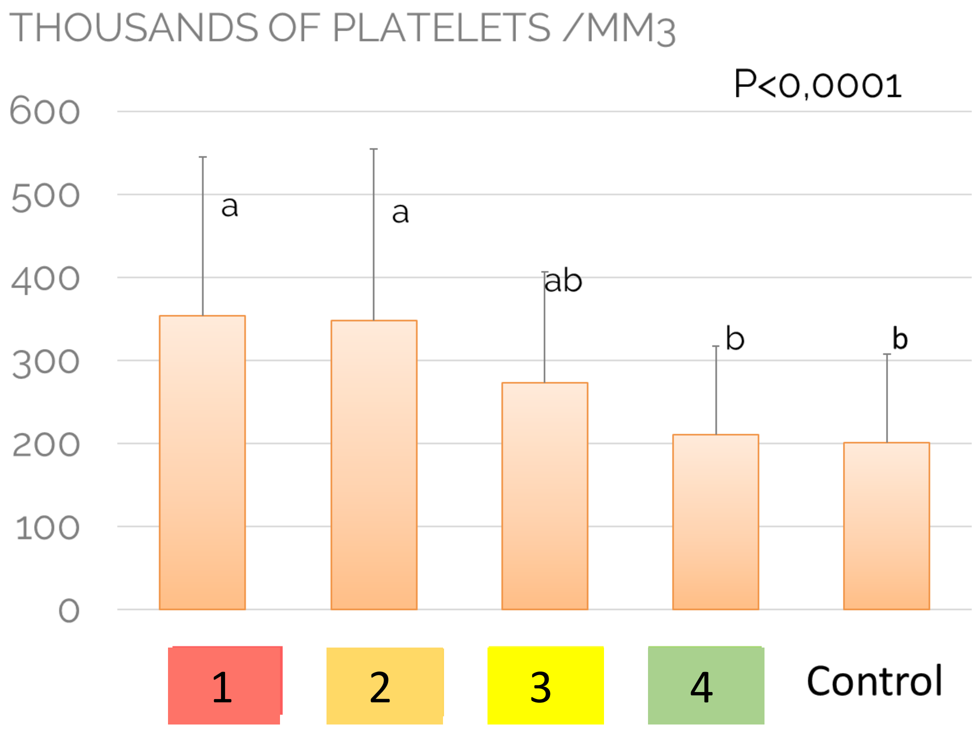
# Number of red blood cells per resistance score




Resistant, Highly resistant and Control rabbits have a significantly higher red blood cells count

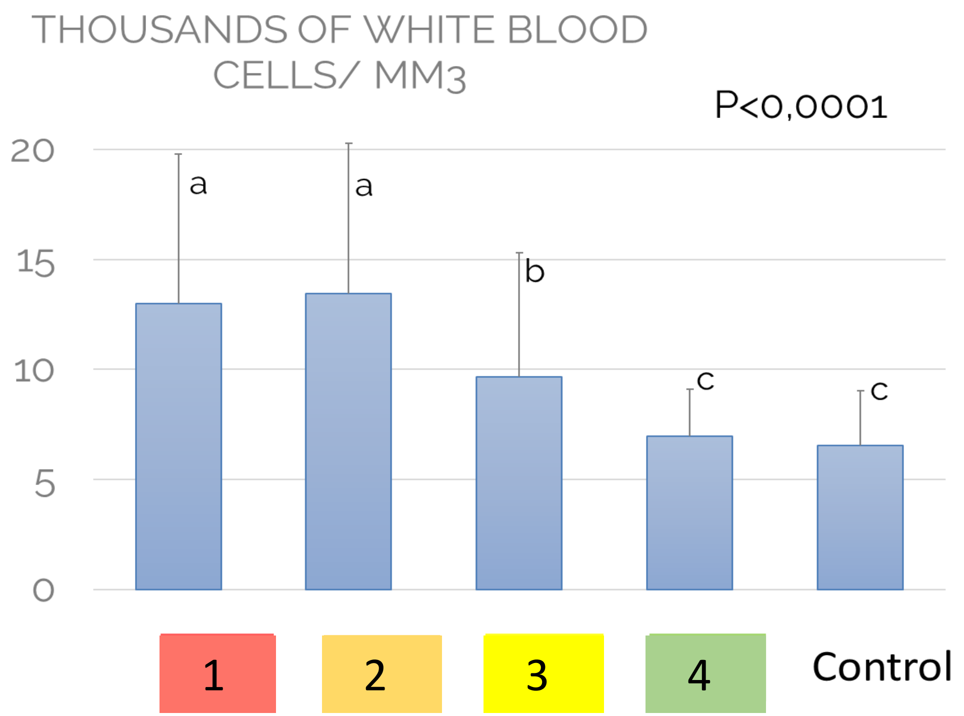
=> hemolytic and hemorrhagic activity of *Pm* toxins in susceptible rabbits

# Number of platelets per resistance score



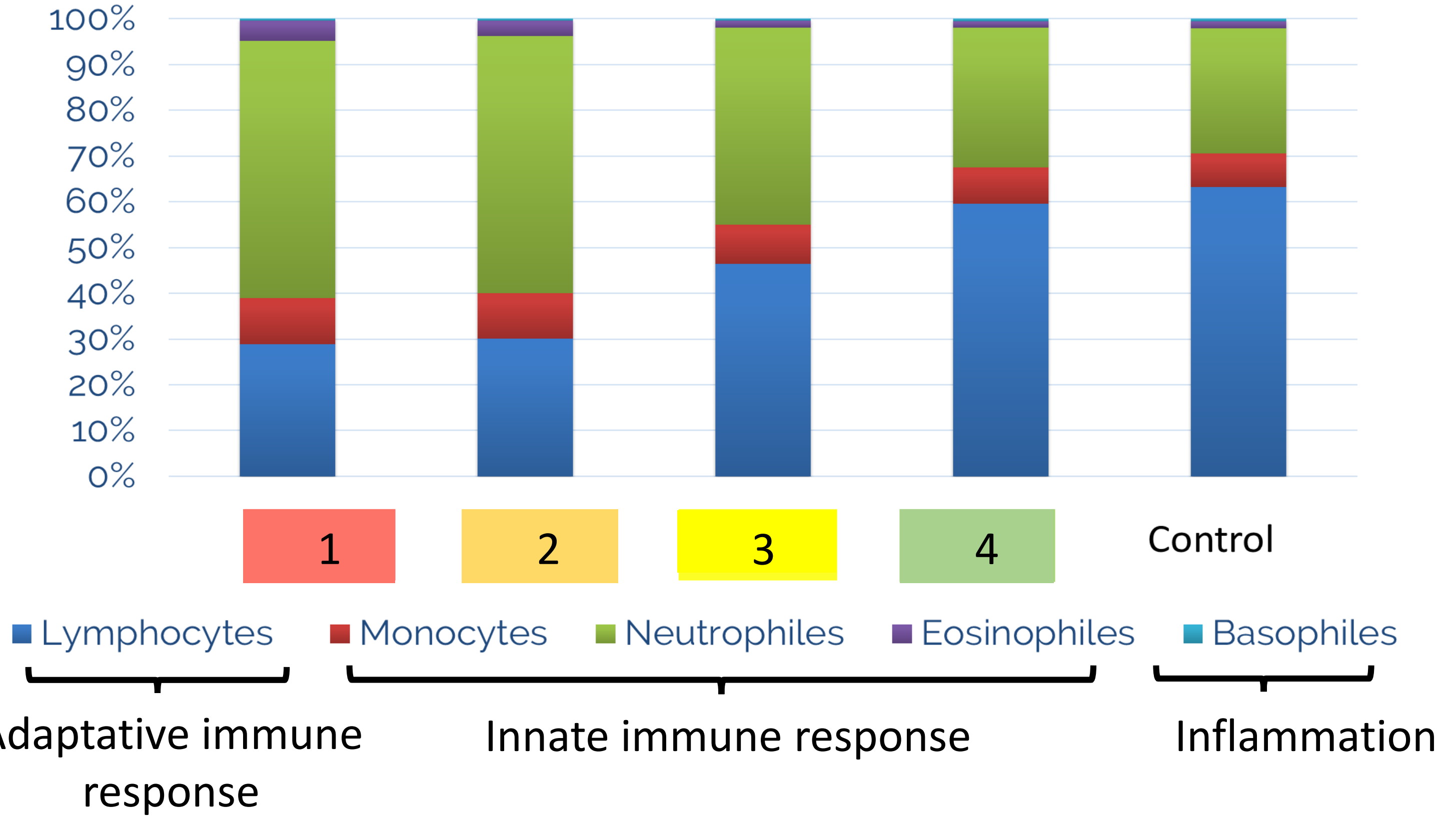

Susceptible animals had a higher level of platelets
=> mobilize more platelets to limit blood loss

# Number of white blood cells per resistance score




Susceptible animals had a higher level of white blood cells
=> involved in the inflammation or in the immune system

# Percentage of white blood cells type per resistance score




Susceptible animals:

- Higher monocytes, neutrophils, eosinophils
- Lower lymphocytes and basophils

# Conclusion

Susceptible rabbits :

- lower red blood cell count and more platelets

  => Hemolytic and hemorrhagic activity of Pasteurella multocida
- higher percentage of monocytes, neutrophils and eosinophils

  => innate immune and inflammatory responses
- lower percentage of lymphocytes (adaptive immune response)

  => do not mount an effective immune response to control the infection.

- Large variability between rabbits in the response to Pasteurellosis infection, which may have a genetic origin

INRAE

# Thank you for your attention

**Gunia M., Lantier F., Bed'hom B., Guitton E., Helies V., Helloin E., Herbert C., 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 | $10^6/mm^3$ | 5.46 ± 0.76 | 5.00 ± 0.71 |
| Hematocrit | % | 35.30 ± 5.12 | 30.61 ± 4.83 |
| Hemoglobin | g/dl | 11.89 ± 1.61 | 10.52 ± 1.41 |
| Lymphocytes | % | 61.34 ± 7.20 | 32.46 ± 12.82 |
| Basophils | % | 0.47 ± 0.14 | 0.36 ± 0.14 |
| Neutrophils | % | 26.56 ± 6.29 | 52.00 ± 12.80 |
| Monocytes | % | 7.19 ± 1.60 | 9.51 ± 2.47 |
| Eosinophils | % | 1,48 ± 0,66 | 3.23 ± 3.10 |
| White Blood Cells | $10^3/mm^3$ | 6.53 ± 2.52 | 12.66 ± 6.78 |
| Platelets | $10^3/mm^3$ | 201.02 ± 106.33 | 333.31 ± 199.98 |