

Estimation of Genetic Parameters of Pasteurellosis Resistance in Crossbred Rabbits

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Summary

This study is a first approach to estimate genetic parameters of resistance to Pasteurellosis in an experimental crossbred rabbit population. Novel disease-related traits were the abscess dissemination score, collected post autopsy, a score related to the presence or absence of *Pasteurella multocida* in the organs, and resistance score. For disease-related traits, heritability estimates ranged from 0.09 (± 0.05) to 0.14 (± 0.05). Highest heritability was estimated for the resistance trait. Results for heritability estimates suggest a contribution of a genetic component to Pasteurellosis resistance. Genetic correlations among disease-related traits were strongly positive. Genetic correlations between disease-related traits and average daily weight gain were strongly negative. These results support the implementation of a selection against Pasteurellosis in the French breeding programs for meat rabbits. Further investigations will be performed using additional disease-related and production traits.

Keywords: pasteurellosis, heritability, genetic correlation, disease resistance, rabbit.

Introduction

Pasteurellosis caused by *Pasteurella multocida* is one of the most common bacterial infection in commercial farms as well as in laboratory rabbits (Coudert *et al.*, 2006). This infection provokes a variety of clinical manifestations such as abscesses, pneumonia, septicaemia, rhinitis, mastitis and pyometra (Coudert *et al.*, 2006). In rabbit meat breeding industry, prevention and control of the spread of the infection is done by maintaining strict environmental hygiene and by using antibiotics. However, use of antibiotics raises a risk of developing antibiotic resistance in bacteria and its' transfer to other species including human.

Hence, a sustainable alternative approach is desirable to overcome drawbacks of such practices and to decrease the prevalence of infection. One approach can be breeding rabbits more resistant to Pasteurellosis. Previous studies suggested possibility of selection for resistance in rabbits, with low to moderate heritability estimates ranging from 0.034 (± 0.006) to 0.28 (± 0.16) (Baselga *et al.*, 1988, Eady *et al.*, 2004, Gunia *et al.*, 2015). Most of these previous studies are based on observable clinical signs of the infection from field data. Such

scenarios possess two drawbacks: i) such approaches might lack accurate diagnosis of the health status which can lead to underestimated heritability estimates (Bishop & Woolliams, 2010). ii) due to uneven exposure to the infection, a rabbit might not show its' true response (potential) which might also lead to inaccurate diagnosis. Such drawbacks can be avoided by analysing different response traits to the infection in a population that is systematically exposed to the pathogen. Hence, in this study we estimated genetic parameters in crossbred rabbits by analysing novel traits from an experimental infection with a strain of *Pasteurella multocida*.

Material 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 PlateForme 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

The study was initiated at an experimental unit, the Pôle Expérimental Cunicole de Toulouse (INRA PECTOUL) with 1,030 crossbred rabbit progenies from six sire lines (two lines each from breeding companies EUROLAP, HYCOLE, and HYPHARM) and one dam line (1777 INRA). This experimental population is representative of the commercial breeding does used by French rabbit breeders. This population was raised in a new building and was checked for being free of usual rabbit pathogens. This study contained on average 171.7 progenies from each sire line (range: 155 - 194). These rabbits were obtained across five batches in one year. The first batch contained 110 rabbits and remaining four batches contained 230 rabbits each. In total 50 rabbits (10 rabbits per batch) were used as controls and 980 rabbits were planned to be inoculated.

The rabbits were transported to the PlateForme d'Infectiologie expérimentale (INRA PFIE) at 36 days of age, one day after weaning. At PFIE, the rabbits were housed in cages (5 animals each) in two rooms with an effort to achieve balanced distribution of genders, paternal, and maternal origins.

Out of 980 rabbits, 16 rabbits died or were euthanized before inoculation, due to digestive disorders (mostly Epizootic Rabbit Enteropathy, ERE). ERE is a potentially fatal gastrointestinal disease with a currently unknown aetiology.

Inoculation and measured traits

Inoculations with *P. multocida* were performed at 42 days of age. 964 rabbits were injected subcutaneously between the shoulder blades with a dose of 8000 bacteria / 0.1 ml saline of the pyrogenic strain LVT62 of *P. multocida*. This strain was previously selected among 160 isolates as virulent and representative of *Pasteurella* field isolates.

Health status of the rabbits was monitored daily for 14 days post inoculation. Body weight was measured a day before inoculation, on 7th and 14th day post inoculation. ADG for the first (adg1) and second (adg2) week post inoculation was then computed as well as the total average daily weight gain (adg-total). During the experiment, 120 rabbits died or were euthanized. Remaining 844 rabbits were euthanized on day 14 post inoculation.

Definition of disease-related traits

The rabbits were autopsied and scored for the number and location of abscesses (Abscess), presence of *P. multocida* in organs and tissues (Bacteria), and resistance (Resistant). The rabbits' body post euthanasia was examined for the signs of Pasteurellosis. Occurrence of abscess was recorded in different regions (inoculation site, front, rear, organs) of the body by the same two scientists during the experiment by using a scoring grid. "0" represents rabbits with no signs of abscess in the body. Scores 1 to 4 represents the location of abscesses from the subcutaneous inoculation site (between the shoulder blades, scored 1) to the whole body, and deep organs (liver, spleen, lung, scored 4).

Spleen, lung, liver and abscess were collected and rapidly frozen. They were kept at 20°C (2-3 months) until they were homogenized then cultured by Touraine Laboratory to identify and quantify *P. multocida*. The tissue samples from liver were used only for the rabbits that died during the experiment. The rabbits were scored for bacterial count from 0 to 4. "0" represents rabbits with no bacterial growth in the culture. Score 1 to 4 represents a spread of bacteria in the body. The rabbits were then scored for resistance to Pasteurellosis from 1 to 5. This composite trait takes into account abscess and bacterial scores. Score 1 represents most resistant rabbits (no signs of abscess and no bacterial growth from tissue homogenates). Scores 2 to 5 represents decrease in resistance to Pasteurellosis.

Statistical analysis

Statistical analysis included 844 rabbits and 109 dead rabbits (out of 120 rabbits) whose death was confirmed due to Pasteurellosis. In total 953 rabbits were used for analysis. The genetic parameters and correlations were estimated for the traits of interest using linear models with ASReml software (Gilmour *et al.*, 2002). Fixed effects tested for each trait were: gender (2 levels), batch (5 levels), weaning age (4 levels), parity of dam (6 levels), and signs of ERE (3 levels). Random effects included in the model were environmental and genetic effects. The litter and interaction of batch, room, and cage (BRC) were included as environmental random effects. Animal component and maternal genetic component were included as random genetic effects. The sire and dam line information was introduced as genetic groups in the pedigree. Log likelihood values obtained from ASReml were used to perform a likelihood ratio test in statistical package R (R Core Team, 2015) to test the significance of the random effects.

The genetic parameters were estimated from univariate analysis of each trait taking into account only significant fixed effects and random effects. Then correlations between different traits were estimated using classical two traits analysis.

Results and Discussions

Out of 953 rabbits used in the analysis, 72 rabbits didn't show any abscess, 79 rabbits didn't show any bacterial growth in tissue samples, and 71 rabbits were resistant to Pasteurellosis (neither abscess nor bacterial growth).

Out of different effects tested in the model, the significant fixed effects are mentioned in Table 1. Rabbits severely affected by ERE (group 2) showed large number of *P. multocida* in their tissues, and severe abscesses. They showed lower resistance to Pasteurellosis than rabbits not affected by ERE. This suggests a possible interaction between Pasteurellosis and

ERE. Breeding for Pasteurellosis resistance might aid indirectly in breeding for ERE resistance as well. Rabbits less severely affected by ERE (group 1) showed higher resistance to Pasteurellosis and lower bacterial count than rabbits without ERE. Rabbits severely affected by ERE (group 2) had the least average daily gain for all three traits *adg1*, *adg2*, and *adg-total*. For all traits, BRC and maternal genetic component were not significant. Litter showed significant effect only for abscess.

Heritability and Correlations:

Heritability estimates for disease-related traits and ADG traits are mentioned in Table 1. For disease-related traits, heritability estimates ranged between 0.09 (± 0.05) and 0.14 (± 0.05). Our heritability estimates are similar to the study performed by Baselga *et al.*, (1988) where fresh lung lobes were examined post euthanasia, and scored based on the extensions of the lesions. Our heritability estimates are higher compared to the study performed by Eady *et al.*, (2004), Eady *et al.*, (2007), and Gunia *et al.*, (2015). This might be due to a systematic exposure of infection in our rabbit population and due to different diagnostic approaches. The diagnostic approach in our study provide us with the “true *Pasteurella* infection” status of each rabbit and estimates close to true genetic variations in the population. The healthy rabbits in our study do not show any sign of Pasteurellosis internally. This information as well as some cases may be missed out when only visible signs are taken into consideration.

In our study, highest heritability estimate of 0.14 (± 0.05) was observed for resistant trait. This suggests that a selection of rabbits based on this combined score could be more efficient than a selection based on a single trait. However, this might be expensive for a breeding program. Hence, one approach to decide which disease-related trait should be considered to breed against Pasteurellosis could lie in calculating correlations between visible signs of Pasteurellosis and clinical signs obtained post euthanasia.

Correlation estimates are presented in Table 2. Positive genetic and phenotypic correlations were observed among disease-related traits and among ADG traits. Disease-related traits showed high genetic correlations amongst themselves and comparatively low phenotypic correlations. Correlation estimates between abscess and bacterial count was inconclusive, as the genetic variance was not estimated properly (not positive definite) although our model converged.

Negative correlations were observed between disease-related traits and ADG traits, as expected. Such negative genetic correlations between disease-related traits and body weight related traits have been reported by previous studies (Eady *et al.*, 2004, Gunia *et al.*, 2015). Such negative genetic correlations are favourable and encourage considering breeding against Pasteurellosis alongside breeding for growth related traits in rabbit populations (Gunia. *et al.*, 2015). Correlations of the disease-related traits with *adg1* and *adg-total* were stronger as compared to those that relate to *adg2*. This might be due to difference in response to infection in different time periods. The rabbits had stronger impact by infection in the first week of the experiment as compared to the second week.

However, inferences of genetic correlations should be investigated with caution. Various biological mechanisms such as pleiotropism, linkage disequilibrium, non-additive genetic effects, dominance, epistatic effects influence genetic correlations (Carey, 1988). Better understanding of such underlying biological mechanisms between traits of interest supplements better decisions in breeding program.

Our study presents a preliminary approach towards an estimation of genetic parameters for resistance to experimental infection with *Pasteurella*. It provides evidences for a

contribution of genetic components from the novel disease-related traits recorded in this crossbred rabbit population. The heritability estimates of these novel traits suggest their potential use as selection criteria for resistance against Pasteurellosis in rabbits. Further analyses using additional disease-related traits (temperature, mortality) and production traits are on the way.

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Table 1. Number of animals scored, mean values and standard deviation (in parantheses) of traits, significant fixed effects, estimated heritability (h^2), standard error (in parantheses) and significant random effect for each analysed trait.

Trait	Number of animals	Mean (SD)	Significance of fixed effects			h^2 (SE)	Litter (SE)
			ERE	Batch	Batch.ERE		
Resistant	951	3.19 (0.94)	**	ns	**	0.14 (0.05)	ns
Abscess	953	2.61 (1.02)	**	ns	ns	0.11 (0.06)	0.09 (0.04)
Bacteria	951	1.36 (0.96)	**	ns	**	0.09 (0.05)	ns
adg1	902	2.94 (18.23)	*	**	ns	0.28 (0.07)	ns
adg2	852	1.98 (17.89)	*	ns	ns	0.19 (0.06)	ns
adg-total	844	1.14 (14.28)	*	**	ns	0.28 (0.07)	ns

** P-value < 0.001, * P-value < 0.05, ns: Not significant (P-value > 0.05)

Table 2. Genetic (above diagonal), phenotypic (below diagonal) correlations and standard errors (in parentheses) among disease-related and production traits.

	Resistant	Abscess	Bacteria	adg1	adg2	adg-total
Resistant		0.99 (0.13)	0.96 (0.08)	-0.99 (0.07)	-0.68 (0.15)	-0.95 (0.05)
Abscess	0.80 (0.01)		NE	-0.99 (0.10)	-0.85 (0.14)	-0.99 (0.14) ¹
Bacteria	0.84 (0.01)	NE		-0.93 (0.17)	-0.71 (0.18)	-0.93 (0.10)
adg1	-0.69 (0.02)	-0.67 (0.02)	-0.51 (0.03)		0.46 (0.18)	0.92 (0.04)
adg2	-0.55 (0.03)	-0.54 (0.03)	-0.56 (0.03)	0.31 (0.03)		0.80 (0.08)
adg-total	-0.79 (0.01)	-0.48 (0.03) ¹	-0.71 (0.02)	0.84 (0.01)	0.77 (0.02)	

NE- Non estimable, ¹: Values obtained by log transformation of adgtotal.