Phenotyping and selecting for genetic resistance to gastro-intestinal parasites in sheep: the case of the Manech French dairy sheep breed

J.M. Astruc1, F. Fidelle2, C. Grisez3, F. Prévot3, S. Aguerre4, C. Moreno4 and P. Jacquiet8

1Institut de l’Elevage, 31321, Castanet-Tolosan, France
2CDEO, Quartier Ahetzia, 64130, Ordiarp, France
3GenPhySE, Université de Toulouse, INPT, INPT-ENVT, 31320, Castanet-Tolosan, France
4INRA-ENVT UMR1225, 31076, Toulouse, France

The genetic selection of sheep for a better resistance to gastro-intestinal nematodes is a challenge for sustainable production on pastures, especially to face the increasing resistance to anthelmintic treatments and to limit these treatments in order to mitigate the chemical releases on the environment. A strategy of phenotyping resistance to parasites has been elaborated in sheep in the veterinary school of Toulouse in France, based on an original experimental design. Young rams are experimentally infected twice with a given dose of larvae of the nematode Haemonchus contortus. Measures of fecal egg counts and hematocrit level 30 days after infection respectively assess the resistance to parasites and control the resilience to infestation. So far, few French sheep breeding programs have experimented this design of phenotyping. In this study, we considered 450 AI rams of the Breed Society of the Blond-Faced Manech dairy sheep breed, phenotyped for parasitism resistance from 2008 to 2015. We firstly aim at describing the experimental protocol and underlining the main results, interests and limits of the phenotyping. Then, the genetic valorization of the phenotypes is presented, through the estimation of genetic parameters and the genetic evaluation proposed to the Breed Society. Finally, strategies and perspectives to include selection for resistance to parasites in the breeding program are considered, especially as genomic selection will offer greater selection efficiency in the upcoming years.

Keywords: gastro-intestinal parasites, genetic resistance, phenotyping, genetic parameters, genetic evaluation, sheep

Gastrointestinal parasites infections are a major health problem for small ruminants grazing on pastures, especially in sheep raised in oceanic climatic conditions, as it is the case for the dairy sheep Blond-Faced Manech, which will be studied in this communication. The economic concern is mainly due to the decrease in production, undesired culling and cost of anthelmintics treatments. The increasing resistance to anthelmintics molecules has lead in numerous flocks to a lack of effect of any treatment, especially when most of them are prohibited during the lactation. The environment issue is also of high concern with the ecotoxicity of the chemical residues resulting in pollution of soil and destruction of the entomofauna. Several alternatives to systematic
treatments have been explored, such as selective treatments, supplementation with condensed tannins or grazing on pastures with tanniferous plants. Genetic selection is also among these promising and sustainable methods to reduce parasitic pressure and increase resistance of the sheep.

The gastrointestinal nematodes (GIN) are internal parasites present in sheep, with different stages of development in their life cycle. The parasitic phases happen inside the host (the sheep), which eats third stage larvae. This larvae mature and develop into adult worms inside the sheep. Adult female worms in the sheep lay eggs in the abomasum or the small intestine. The eggs are then spread in the outdoor environment through the feces and hatch into first-stage larvae which give different stages of larvae in the pastures. Third-stage larvae (L3) migrate on to the herbage and are ingested by grazing sheep. The most frequent GIN in the area of the Blond-Faced Manech breed are *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*.

The Blond-Faced Manech is a dairy sheep breed reared mainly outdoor on pastures, in the French Basque country, in the Pyrenean Mountains of south-western France. It is a rainy and mountainous area, favorable to GIN. The total population of the breed reached 280,000 ewes in 2015 (Arranz, personal communication). An efficient breeding program is conducted by the CDEO breed organization, based on a selection nucleus representing 28% of the whole population and on the progeny-test of 150 rams by artificial insemination each year. The traits included in the selection criterion are so far milk yield (MY), fat (FC) and protein (PC) content and somatic cell score (SCS). As resistance to anthelmintics is spreading in the Pyrenean area, the CDEO decided to experiment a genetic selection for resistance to parasites, in collaboration with the veterinary school of Toulouse (ENVT), INRA and the French Livestock Institute (IDELE).

The more common indicator for resistance to parasites is the Fecal Egg Count (FEC), expressed in eggs per gram of feces, from a coproscopy (Jacquiet et al., 2011). FEC is therefore commonly considered as the reference method to assess the host resistance. It measures the capacity of the sheep to decrease the establishment, the development, the fecundity and the fitness of the worms. In case of hematophagous parasites, such as *Haemonchus contortus*, the packed cell volume (PCV) may also be measured, with two benefits. It first allows to verify the fitness of the sheep after an infestation. But it also assesses the resilience of the sheep, i.e. its ability to maintain its performance while subjected to a parasitic challenge. As a drawback, individual FEC is costly and time-consuming, requiring a sample of feces, a specific preparation of the feces on the lab and finally a count of the eggs on microscope.

Many countries and breeds, especially in Australia, New Zealand, UK, Uruguay (Morris et al., 2010; Ciappesoni et al., 2010; Woolaston et al., 2001) measure resistance to parasites in situation of natural infestations on pastures. But these measures are dependent on meteorological conditions. That is why, in France, an original design has been set up, consisting in experimental infestations applicable to young rams gathered in breeding or AI centers. These rams are genetically important because they are the future sires of the breed.

The figure 1 summarizes the main phases of the experimental protocol of infestation, conceived in the years 2000 by Gruner for experimental purposes (Gruner et al., 2004a) and improved by Jacquiet for selection purposes (Jacquiet et al., 2015). It is based on two successive infestations of a given and known dose of L3 of *Haemonchus contortus* (hematophagous GIN). At time 0, rams are given 3500 L3. 30 days later, feces are collected for FEC. A blood sample is also collected to measure PCV, which is compared
to PCV before infection. Rams are then drenched. 15 days later, a second infection with 5000 L3 occurs, with the same set of measures: FEC 30 days after, difference of PCV between after and before second infection.

This protocol has been set up for many years and adapted to each breed and to the age of the rams (this protocol is also applied in INRA experimental flocks and in some meat sheep breed such as the Romane, BMC or Charmoise breeds whose rams are infected earlier in their life, at the age of 4 months), especially regarding the dose of L3. In the case of the Blond-Faced Manech, the infestation occurs when the rams are in lay-off, about 7 months before their first use as service rams. Rams are 2 or 3 years old. The rams had previously entered the breeding center at the age of 2 months, they are raised inside, progeny-tested at the age of either 8-month-old or 20-month-old. At the time of the experimentation, they have never known the pasture and are therefore naïve regarding parasites.

Many studies have assessed the relevance of such an experimental infestation. The main results are the following: there is a high correlation (~0.8-0.9) between resistance in experimental infestation and resistance in natural infestation (Gruner et al. 2004b). The correlation between resistance to Haemonchus contortus vs other species of GIN is high and near to 1 (Gruner et al., 2004a). The correlation between resistance of young rams in breeding center and resistance of offspring on pastures is currently being assessed, through on-going on-farm experiments using divergent lines of rams: the first results are encouraging. Finally, the resilience (PCV) allows to check that rams have no pathologic effects. The AI center also verified that the infestation did not decrease the semen production during the AI period 7 months after the second infestation.

On the whole, 5 experimental infestations were performed from 2008 and 2015 in the CDEO AI center. 451 Blond-Faced Manech rams, mainly aged 2 or 3, were phenotyped. The main measures obtained from the infestations are:

![Figure 1. Protocol of experimental infestation (from Jacquiet et al., 2015)](image)
- FEC1 = FEC at first infestation.
- FEC2 = FEC at second infestation.
- diffPCV1 = PCV1f - PCV1i (PCV just 30 days after first infestation minus PCV just before first infestation).
- diffPCV2 = PCV2f - PCV2i (PCV just 30 days after second infestation minus PCV just before second infestation).

Table 1 shows the average and standard deviation of the different phenotypes over the entire dataset (451 rams).

Figure 2 illustrates how important is the phenotypic variability of the rams regarding to the FEC (in this example: FEC at the second infestation in the 2014 design). For some rams, no eggs were counted in the sample of feces. We can suppose that these rams have succeeded in preventing worms from establishing, multiplying and developing, whereas some others, with FEC exceeded 10,000 eggs per gram, are susceptible to parasites.

Table 1. Descriptive statistics on phenotypes of resistance and resilience analyzed in this study

<table>
<thead>
<tr>
<th>Traits</th>
<th>FEC1</th>
<th>FEC2</th>
<th>diffPCV1</th>
<th>diffPCV2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>2141</td>
<td>1641</td>
<td>3.40</td>
<td>1.00</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>2491</td>
<td>1787</td>
<td>3.75</td>
<td>3.15</td>
</tr>
</tbody>
</table>

Figure 1. Range of FEC in the second infestation of the 2014 protocol, including 132 rams
Genetic parameters of resistance and resilience traits, as well as their standard errors, were estimated using the restricted maximum likelihood estimation with the VCE package.

The model used is the following:

\[ Y_{ijk} = \mu + P_i + A_j + R_k + e_{ijk} \]

Where \( Y_{ijk} \) is the dependent variable (FEC1, FEC2, diffPCV1 and diffPCV2), \( \mu \) is the population mean, \( P_i \) is the fixed effect of protocol \( i \) (representing the contemporary group, one per year), \( A_j \) is the fixed effect of age \( j \) of the ram, \( R_k \) is the additive genetic random value of the ram \( k \) and \( e_{ijk} \) is the random residual effect. The rams were submitted to only one protocol in their lifetime. There were no repetition and therefore no permanent environmental random effect.

For genetic analysis, the traits of resistance are the square root of FEC1 and FEC2. The square root transformation must be performed because of positively skewed distribution of the FEC. Such a transformation results in more symmetrical distributions.

All rams are born from known sires. There were on average 4.5 rams per sire, ranging from 1 to 17.

Genetic correlation between resistance traits and other traits included in the selection criterion of the Blond-Faced Manech were also estimated, using the same REML from VCE package. The traits taken into account were milk yield (MY), fat (FC) and protein (PC) content, and somatic cell score (SCS). The model for these traits were:

\[
MY_{ijkr} = \mu + HY_i + A_j + M_k + I_l + R_r + e_{ijkr} \\
FC_{ijmr} = \mu + HY_i + A_j + C_m + R_r + e_{ijmr} \\
PC_{ijmr} = \mu + HY_i + A_j + C_m + R_r + e_{ijmr} \\
SCS_{ijkr} = \mu + HY_i + A_j + M_k + R_r + e_{ijkr}
\]

Where \( HY_i \) is the fixed effect of flock x year \( i \), \( A_j \) is the fixed effect of age at first lambing \( j \), \( M_k \) is the fixed effect of month at lambing \( k \), \( C_m \) is the fixed effect of combination of sampling \( m \), \( I_l \) is the fixed effect of interval between lambing and first test-day \( l \), \( R_r \) is the additive genetic random value of the ram \( r \) and \( e_{ijkr}, e_{ijmr}, e_{ijmr}, e_{ijkr} \) are the random residual effect for respectively the trait MY, FC, PC, SCS. The edited file for MY, FC, PC and SCS traits were constituted of 41,543 ewes in first lactation with performances in years 2014-2016.

Table 2 gives the main genetic parameters among resistance and resilience traits.

Heritability of FEC is different from first and second infestation: it is low for first infestation and moderate for second infestation. However, the genetic correlation is high and positive between FEC at both infestation. This suggests that measuring only the second infestation could be relevant on a genetic point of view.

Heritability of resilience seems to be the same at both infestations, around 0.2. However, the standard error is high, especially for the second infestation. Further results from new experimental infestations in the next years should consolidate the results. The negative correlation between diffPCV at both infestations is surprising and suggests
that the traits are not really the same. An interpretation could be that the first infestation of naïve rams produces parasite-specific antibodies, whereas the second infestation leads to acquisition of effective immune response with a genetic control different.

The genetic correlation between resistance and resilience at the first infestation is high and positive. Whereas the same genetic correlation at the second infestation is near to zero. This is in accordance with the fact that the resilience at the first and the second infestation are not under the same genetic control.

Table 2. Genetic parameters of resistance and resilience traits.

<table>
<thead>
<tr>
<th></th>
<th>FEC1</th>
<th>FEC2</th>
<th>diffPVC1</th>
<th>diffPVC2</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC1</td>
<td>0.095 ± 0.052</td>
<td>+0.918 ± 0.072</td>
<td>+0.931 ± 0.096</td>
<td></td>
</tr>
<tr>
<td>FEC2</td>
<td>+0.295</td>
<td>0.322 ± 0.069</td>
<td>0.210 ± 0.072</td>
<td>-0.042 ± 0.234</td>
</tr>
<tr>
<td>diffPVC1</td>
<td>+0.256</td>
<td>0.210 ± 0.072</td>
<td>-0.493 ± 0.255</td>
<td></td>
</tr>
<tr>
<td>diffPVC2</td>
<td>-0.004</td>
<td>0.230 ± 0.167</td>
<td></td>
<td></td>
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</tbody>
</table>

Heritabilities on the diagonal; genetic correlations above the diagonal; phenotypic correlations below the diagonal.

With respects to the difficulty to phenotype resistance to GIN as it is costly and time-consuming, the previous results are interesting. As the heritability of resistance at the second infestation is moderate and superior to the one at the first infestation and given the genetic correlation between both measures, it should be cost-effective to measure individuals at the second infestation only, even though the two successive infestations of the protocol are necessary.

The genetic correlation between FEC at the second infestation and milk yield (which is the main production criteria, weighing for 30% in the selection criteria) is slightly unfavorable (+0.21). This means that an efficient selection on dairy potential selection might have increased the susceptibility of the Manech breed to the infections of GIN. The genetic correlation between FEC and milk components shows a slightly unfavorable relationship with fat content (+0.21) and a slightly favorable relationship with protein content (-0.19). Finally, the genetic correlation between FEC and somatic cell count suggests a slightly unfavorable relationship (-0.18). Data are reported in Table 3.

We must be very careful with these results of correlations. They are based on a data set of only 451 rams phenotyped for resistance to parasitism. The standard error shows that they may be very near to zero. Nevertheless these results are consistent with the lack of clear correlation between resistance to parasitism and production traits, estimated mainly in sheep population specialized for meat and/or wool production.

Table 3. Genetic correlations between resistance trait at the second infestation and milk yield (MY), fat (FC) and protein (PC) contents and somatic cell score (SCC).

<table>
<thead>
<tr>
<th></th>
<th>MY</th>
<th>FC</th>
<th>PC</th>
<th>SCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEC2</td>
<td>0.211 ± 0.121</td>
<td>+0.215 ± 0.130</td>
<td>-0.188 ± 0.110</td>
<td>-0.178 ± 0.116</td>
</tr>
</tbody>
</table>
(Bishop et al., 2007). In dairy sheep, few publications exist (Gutiérrez-Gil et al., 2010, Sechi et al, 2009). In Gutiérrez-Gil et al. (2010) the genetic correlation reported between FEC and MY ranged from -0.08 to -0.18 in situation of natural infestation of dairy Churra ewes. Sechi et al. (2009) found a moderately favorable genetic correlation between FEC and SCS in situation of natural infestation of dairy Sardinian x Lacauine backcross Sarda ewes. In both cases, as in the current study in Manech breed, the correlations are slight, either favorable or unfavorable, but basically circa zero.

A genetic variability of resistance to nematodes were exhibited in an experimental and controlled challenge, rendering selection feasible. Unfavorable correlation between resistance and milk yield suggests that resistance to GIN might be considered in the selection, even though the reliability of the results prevents from drawing definitive conclusion.

Phenotyping resistance to nematodes is laborious and expensive. Two ways to reduce costs and work are currently assessed: concerning FEC measure, a more automatic method based on quantitative real-time PCR from worm DNA is investigated; in addition, it is useful to consider the decrease of number of individual FEC: measuring FEC2 only, which presents a moderate heritability, but a strong correlation with FEC1 is a way to explore.

Two strategies of selection are to be considered in parallel: on the short-term, using AI resistant rams should be useful for flocks presenting high level of resistance to anthelmintics. On the long-term, a classical selection with selection pressure on rams in breeding/AI center must be thought over. For these purposes, a genetic evaluation has been performed for 2 years and EBVs have been provided to Blond-Faced Manech breed society.

From an ICAR point of view, resistance to nematodes in sheep is a novel trait which should be dealt with by the ICAR Guidelines on sheep. Recommendations to measure this resistance and the associate traits such as resilience could be proposed both under natural conditions and experimental conditions. The new ICAR working group on sheep, goat and small camelid must be a relevant forum to achieve this task.

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We also thank the CDEO (breeding organization of Blond-Faced Manech) for providing samples, as well as for their motivation and enthusiasm regarding difficult-to-measure novel traits.

We finally address a special thanks to Philippe Jacquiet and his team from ENVT (Veterinary School of Toulouse) who performed the infestation, the biological sampling and the on-lab phenotyping.
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List of references


