

Using antibody response to Maedi Visna Virus as selection criterion in sheep

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Maedi Visna

Maedi Visna (MV) in sheep is caused by a small ruminant lentivirus and it is associated with progressive and persistent inflammatory lesions in the lungs, udder, joints and central nervous system.

Infected animals may remain asymptomatic carriers even for several years and only a portion of them will display clinical signs.

The economic impact of MV virus (MVV) infection is not yet fully assessed but several authors reported increased culling and replacement rates as well as decreased milk production and lamb growth

Infection occurs either as a lamb during the suckling period through the colostrum of an infected dam or later in life through the inhalation of respiratory secretions in prolonged close contact.

There are no effective drugs or vaccines to treat or prevent MVV infection

Control plans are costly and laborious since they rely on identifying and eliminating infected animals

Sardinian context

The estimated prevalence of MV in sheep in Sardinia is high: 90% at flock level and 35% at individual level.

Control programs based on eliminating infectious animals are challenging.

The viral strain causing MV in sheep is also responsible for caprine arthritis encephalitis (CAEV), causing many outbreaks in Sardinia where there are promiscuous sheep and goat flocks.

In this context, selective breeding could be a potential effective approach either to avoid costly and laborious control plans or reduce the frequency of CAEV outbreaks in the goat population

Selective breeding needs to identify appropriate selection criteria and design specific recording protocols

For most infectious diseases, antibody response determined through Enzyme-linked immunosorbent assay (ELISA) test is used for prevalence studies and designing control and/or eradication plans.

Individual infectious status (IS) to MV is usually determined through the level of the antibody response to ELISA test with cut-off values supplied by the manufacturer's guidelines.

The accuracy of ELISA test relies on its specificity and sensitivity.

Moreover, individuals can be infected by MVV or seroconvert late in their productive life.

AIM

To verify if the IS determined by ELISA tests routinely performed to assess MV prevalence may be used for selective breeding by estimating heritability in an experimental flock of Sarda ewes using different models to manage the potential bias generated by the age at the ELISA test

Materials and methods

The trial was conducted in an experimental flock of Sarda ewes located in the south of Sardinia. The annual average size was approximately 800 ewes with a replacement rate of around 25%.

The flock has been managed with the seasonal farming system commonly adopted by commercial farms in Sardinia, based on grazing natural or cultivated swards and supplements of hay, silage and concentrate.

No biosafety measures have been adopted, and then MVV transmission might have occurred either as a lamb during the 30d suckling period or later since ewes have been kept in groups and housed in common pens before the 2 daily milkings and during the night or adverse weather conditions.

Data

Antibody response was assessed through ELISA test (kit ELISA IN3 Diagnostic Screening).

The **individual infectious status (IS: 0 for non infected and 1 for infected ewes)** was assigned according to a cut-off value of the antibody levels based on the corrected optical density ratio of sample to positive control.

First sampling was carried out in 2016 on 841 young and adult ewes.

Thereafter, periodic samplings were carried out approximately every six months until 2020.

Ewes resulting IS = 1 were not re-tested assuming specificity of 100%

Moreover, 676 ELISA tests carried out in 2022 on the whole flock were included in the analysis

Finally, a total of 3,215 ELISA tests were carried out on 2,276 ewes

Date of sampling	N samples
2016 June	841
2017 January	407
2017 July	130
2018 January	291
2018 July	189
2019 January	280
2019 July	100
2020 January	301
2022 October	676
Total	3215

Phenotype

A single IS per ewe (2,276 IS in total) corresponding to the last IS = 0 or the first IS = 1 test in the ewe's lifetime was used as phenotype for the genetic analysis.

Genotyping

All the ewes (2,276) and their ancestors (331 sires and 1,268 dams) were genotyped with the Illumina OvineSNP50 Beadchip (3,544 in total)

Genetic analyses

Two linear genetic models were compared for the ability to account for the age of the ewes at the retained IS

- **Model 1:** age adjustment in the model
- **Model 2:** pre-adjustment and weight of IS = 0 records according to the age

Model 1: age class in the model

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{X}\mathbf{b} + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

where

$\mathbf{y}$ was the vector of retained IS corresponding to the last IS = 0 or the first IS = 1 test in the ewe's lifetime (binary variable); $\mathbf{b}$ was the fixed effect of the age class at the retained ELISA test (3 levels); $\mathbf{a}$ was the vector of random additive genetic effect; $\mathbf{e}$ was the vector of random residuals. $\mathbf{X}$ and $\mathbf{Z}$ were the incidence matrices relating $\mathbf{y}$ to $\mathbf{b}$ and $\mathbf{a}$. Vector $\mathbf{a}$ was assumed to be distributed as $\mathbf{a} \sim N(0, \mathbf{H}\sigma_a^2)$ where σ_a^2 was the additive genetic variance, and $\mathbf{H}$ was the realized relationship matrix (blending: 0.95 $\mathbf{G}$; 0.05 $\mathbf{A}$). The pedigree relationship matrix ($\mathbf{A}$) was constituted of 11,223 animals tracing back 10 generations from the phenotyped ewes. The genomic relationship matrix ($\mathbf{G}$) was constituted of 3,544 animals. Vector $\mathbf{e}$ was assumed to be distributed as $\mathbf{e} \sim N(0, \mathbf{I}\sigma_e^2)$ where σ_e^2 was the residual variance and $\mathbf{I}$ was an identity matrix.

The genetic analysis was run using BLUPf90+ software.

Age class	IS = 0	IS = 1	Total
1: $x \leq 20$ mo (approximately the age at first lambing);	209	651	860
2: $21 \leq x \leq 31$ mo (approximately the age at second lambing);	103	375	478
3: $32 \text{ mo} \leq x$ (approximately the age at third lambing and over);	191	747	938
Total	503	1773	2276

Average IS per age class are not estimations of the corresponding prevalence since they are affected by the moment during the lifetime where the retained test was performed. For instance: ewes IS = 0 early in life could be infected or seroconvert later in the ewe lifetime biasing the prevalence at the first age class

Model 2: pre-adjustment and weight of records IS = 0 according to the age class

Step 1: Prediction of the prevalences in the 3 age classes

P_1 = the observed frequency of IS = 1 among ewes with ELISA test in the age class 1 = $572/1282 = 0.45$

Probability to turn from IS = 0 at age class 1 to IS = 1 at age class 2

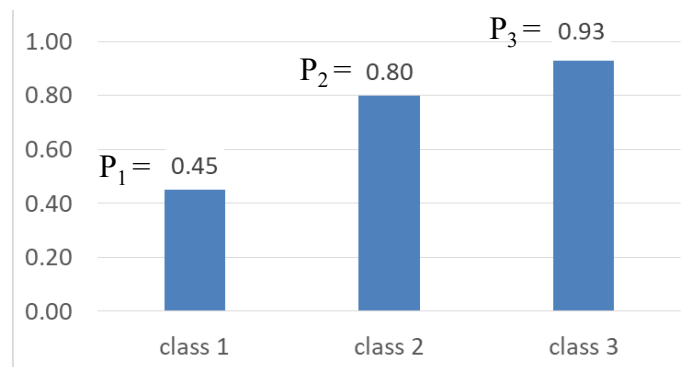
P_{1-2} = 186 ewes with IS = 1 in age class 2 out of 293 with IS = 0 in age class 1 and a successive ELISA test in age class 2: $186/293 = 0.63$

Probability to turn from IS = 0 at age class 2 to IS = 1 at age class 3

P_{2-3} = 58 ewes with IS = 1 in age class 3 out of 90 with IS = 0 in age class 2 and a successive ELISA test in age class 3: $58/90 = 0.64$

P_2 = predicted prevalence of IS = 1 in age class 2 = $P_1 + (1 - P_1) * P_{1-2} = 0.45 + 0.55 * 0.63 = 0.80$

P_3 = predicted prevalence of IS = 1 in age class 3 = $P_2 + (1 - P_2) * P_{2-3} = 0.80 + 0.20 * 0.64 = 0.93$



The predicted prevalence in age class 3 represents the **reference prevalence for pre-adjustments**

Model 2: pre-adjustment and weight of records IS=0 according to the age class

Step 2: Calculation of pre-adjustments of IS = 0 at age class 1 and 2

Pre-adjusted values:

IS = 0 in age class 1 was pre-adjusted for the probability to become IS = 1 in age class 3 (Y_{adj_1})

$$Y_{adj_1} = P_{1-2} + (1 - P_{1-2}) * P_{2-3} = 0.63 + (1 - 0.63) * 0.64 = \mathbf{0.87}$$

IS = 0 in age class 2 was pre-adjusted for the probability to become IS = 1 in age class 3 (Y_{adj_2})

$$Y_{adj_2} = P_{2-3} = \mathbf{0.64}$$

Model 2: pre-adjustment and weight of records IS=0 according to the age class

Step 3: Calculation of the weight of pre-adjusted records

Pre-adjusted records were weighed for their accuracy

$$w_i = \sqrt{1 - \frac{MSE_i}{MST}}$$

where

MST was calculated as the mean square deviation of 0 and 1 records from reference prevalence (P_3)

$$\begin{aligned} MST &= (0 - P_3)^2 * (1 - P_3) + (1 - P_3)^2 * P_3 = \\ &= (0 - 0.93)^2 * 0.07 + (1 - 0.93)^2 * 0.93 = 0.0651 \end{aligned}$$

and

MSE was calculated as the mean square deviation of **pre-adjusted records** at age class 1 or 2 from 0 or 1 considering the reference prevalence:

$$\begin{aligned} MSE_1 &= (Y_{adj1} - 0)^2 * (1 - Y_{adj1}) * (1 - P_1) + (Y_{adj1} - 1)^2 * Y_{adj1} * (1 - P_1) + (1 - 1)^2 * P_1 \\ &= (0.87 - 0)^2 * (1 - 0.87) * (1 - 0.45) + (0.87 - 1)^2 * 0.87 * (1 - 0.45) + (1 - 1)^2 * 0.45 = 0.0622 \end{aligned}$$

$$\begin{aligned} MSE_2 &= (Y_{adj2} - 0)^2 * (1 - Y_{adj2}) * (1 - P_2) + (Y_{adj2} - 1)^2 * Y_{adj2} * (1 - P_2) + (1 - 1)^2 * P_2 \\ &= (0.64 - 0)^2 * (1 - 0.64) * (1 - 0.80) + (0.64 - 1)^2 * 0.64 * (1 - 0.80) + (1 - 1)^2 * 0.80 = 0.0461 \end{aligned}$$

$$w_1 = \sqrt{1 - \frac{0.0622}{0.0651}} = 0.21 \quad w_2 = \sqrt{1 - \frac{0.0461}{0.0651}} = 0.54$$

All other records were assigned a weight of 1.

Model 2: pre-adjustment and weight of records IS=0 according to the age class

$$\mathbf{y} = \mathbf{1}\mu + \mathbf{Z}\mathbf{a} + \mathbf{e}$$

where

$\mathbf{y}$ was the vector of pre-adjusted IS (Yadj) - 4 values (0; 0.87; 0.64; 1); $\mathbf{a}$ was the vector of random additive genetic effects; $\mathbf{e}$ was the vector of random residuals. $\mathbf{Z}$ was the incidence matrix relating $\mathbf{y}$ to $\mathbf{a}$. Vector $\mathbf{a}$ was assumed to be distributed as $\mathbf{a} \sim N(0, \mathbf{H}\sigma_a^2)$ where σ_a^2 was the additive genetic variance, and $\mathbf{H}$ was the realized relationship matrix (blending: 0.95 $\mathbf{G}$; 0.05 $\mathbf{A}$). The pedigree relationship matrix ($\mathbf{A}$) was constituted of 11,223 animals tracing back 10 generations from the phenotyped ewes. The genomic relationship matrix ($\mathbf{G}$) was constituted of 3,544 animals. Vector $\mathbf{e}$ was assumed to be distributed as $\mathbf{e} \sim N(0, \mathbf{R}^{-1}\sigma_e^2)$ where σ_e^2 was the residual variance, and $\mathbf{R}$ was a diagonal matrix with phenotype weight (0.21; 0.54; 1) as diagonal elements.

The genetic analysis was run using BLUPf90+ software

Results

Data used in the genetic models

	Age class			
IS	1	2	3	Total
0	209	103	191	503
1	651	375	747	1773
Total	860	478	938	2276
	0.76	0.78	0.80	0.78

The overall prevalence across ages using the retained IS was 0.78 (1773/2276) vs the predicted one of 0.93

Underestimation due to the presence of young ewes not retested later in the lifetime

Variance components estimates and heritability

	s^2_a	s^2_e	s^2_{tot}	h^2	s.e.	Model
Model 1	0.069	0.083	0.152	0.454	0.033	$Y(0 \text{ and } 1) = \text{age class} + a + e$
Model 2	0.036	0.043	0.079	0.458	0.037	$Y_{adj}(0; 0.64; 0.87; 1) = a + e(\text{weight})$

- Heritability was high for both models
- Pre-adjusting and weighing records $IS = 0$ reduced total and genetic variances by 47% suggesting that using raw IS records inflates variances.
- The solutions for age class in model 1 are inconsistent and not related with the prevalence

Age class solutions of model 1

Age class	solution	S.E.
1	0.85	0.06
2	0.86	0.06
3	0.79	0.06

Mean and correlations of breeding values (GEBV)

IS	Age class	Yadj	N	Model 1	Model 2
0	1	0.87	209	-0.50	-0.07
	2	0.64	103	-0.49	-0.15
	3	0.00	191	-0.39	-0.41
0 Total			503	-0.46	-0.21
1	1	1.00	651	0.04	0.04
	2	1.00	375	0.03	0.04
	3	1.00	747	0.11	0.03
1 Total			1773	0.07	0.04
Total			2276	-0.05	-0.02

Correlation

- between GEBV of the two models was 0.74 (n=2276)
- by age class were:
 - Age class 1= 0.67 (N=860)
 - Age class 2= 0.88 (N=478)
 - Age class 3= 0.96 (N=938)
- ewes in class 1 with IS = 0: 0.43
- ewes in class 2 with IS = 0: 0.78

Average GEBV of ewes with IS = 0 in age class 1 and 2 are lower in model 1 (likely overestimation)

The correlation between GEBV of ewes with IS = 0 in class 1 and 2 were 0.43 and 0.78 respectively, showing that a relevant re-ranking occurs when data are pre-adjusted.

**Comparison of the ranking of 11 sires with at least 10 daughters with IS
and $\geq 50\%$ in class 1 or 2 with IS = 0
(out of 178 sires of ewes with IS)**

ID animal	N of daughters (Nd)	Nd with IS = 0	Nd with IS = 1	Nd with IS Adj	%Nd with IS Adj	Rank model 1	Rank model 2
IT092001484032	38	29	9	19	0.50	2	9
IT092001484037	24	20	4	16	0.67	5	12
IT092001384379	27	20	7	20	0.74	3	28
IT090002208221	24	21	3	21	0.88	1	78
IT090002507868	16	12	4	12	0.75	7	81
IT090002507893	13	12	1	12	0.92	4	83
IT090002507920	16	13	3	13	0.81	6	95
IT090002149230	26	15	11	15	0.58	13	116
IT090002507896	13	8	5	8	0.62	29	132
IT090002507898	11	10	1	10	0.91	10	144
IT090002507915	29	17	12	17	0.59	20	175

1 the most resistant
178 the least resistant

Conclusions

Antibody response to ELISA test can be used in the genetic evaluation for resistance to MVV since it shows quite high heritabilities

Records of ewes with $IS = 0$ must be pre-adjusted and weighed according to the age

- to take into account the probability to be infected or seroconvert later in the lifetime and
- to avoid bias in the breeding values estimates of young animals

Pre-adjustment and weights are specific of breeding systems and populations

Further insights

Several studies proposed different candidate genes associated with resistance to MV, the most promising candidate being the transmembrane protein gene 154 (TMEM154) (Heaton et al., 2012)

"Effect of E35K TMEM154 variants on the genetic variability of antibody response to Maedi Visna Virus in Sarda sheep", Salaris et al, Number 85.44 - Poster Session 85 – this congress

Results showed a strong association between the E35K genotype at the TMEM154 gene and resistance to MV in Sarda breed and an important genetic variability persisting even when the E35K genotype effect is considered in the genetic model.



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