Integrating technologies for the sustainable control of gastrointestinal parasites in sheep: The Argentinean case

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Abstract

Gastrointestinal nematode infections in sheep are a major concern among breeders due to the economic losses they cause in terms of a reduction in both productivity and viability of animals. The situation worsens in face of the emergence of anthelmintic-resistant parasites. In this context, breeding and management practices aimed at an integrated control of parasites, such as raising parasite-resistant sheep, are required. This study focused on the genetic variation underlying parasite resistance in sheep, for potential use in breeding programmes. An artificial challenge with infectious *H. contortus* L_3 was carried out in the northeast region of Argentina for more than 10 years in 1 072 Corriedale lambs with an average age of 5.6 months. Body weight, faecal egg count, packed cell volume, and FAMACHA© score were recorded at different time points post-challenge and their heritability and phenotypic and genetic correlations were estimated. Animals were genotyped on 173 single nucleotide polymorphisms belonging to 77 candidate genes for immune response. The results indicate that there is sufficient genetic variability for the four traits studied, which presented moderate heritabilities (in the range 0.29 to 0.44) and increased along the challenge period, with the exception of the hematocrit, which decreased. Association analyses identified seven markers associated with estimated breeding values for faecal egg count, located in genes involved in different stages of the pathogen-host interaction process. The information obtained supports the potential of marker-assisted breeding schemes to enable profitable and sustainable sheep production.

Keywords

gastrointestinal nematodes, resistance/resilience, variance components, Corriedale sheep, candidate genes

1. Introduction

Gastrointestinal nematodes (GINs) impose severe restrictions on sheep production around the world and are a major cause of economic losses. In the northeast region of Argentina, approximately 2.1 million sheep are raised (14.2 percent of the country's total sheep) in 37 288 productive units (31.6 percent of the total units) and in the central region 2.7 million sheep are raised (18.3 percent of the total for the country) in 45 298 productive units (38.3 percent of the total units). The productive units, mostly made up of family structures, belong to medium and small producers. Breeding is outdoor on natural and/or cultivated pastures (Faverio *et al.*, 2016). The temperature and humidity conditions in both regions are conducive to the development of parasites and the most infective and abundant species is *Haemonchus contortus*.

Although mortality is, in general, the most visible sign of intense parasitism, the loss of body weight in lambs is another consequence, which can reach up to 10 percent. GINs cause reductions of 15–20 percent in wool production, with estimated loss of around USD 2 per animal annually. On average, the death of each reproductive female causes losses of around USD 70 and for each dead ram, USD 400 would be lost (Cetra, B., personal communication).

The common practice for the control of GINs is by means of antiparasitic drugs, which are used several times a year, generally based on the pattern of infestation (Suarez and Busetti, 1995). However, the indiscriminate use of antiparasitic drugs "to clean flocks" without a real notion of key concepts on epidemiology and other complementary measures for control has resulted in the emergence of drug-resistant parasites (Suárez, 2007). Evidence of resistance of parasites to biocidal drugs was shown for the first time in Argentina in the '90s and later, in a study carried out in the province of Corrientes by INTA-FAO (2003–2005), it was shown that 80 percent of the flocks had parasite resistance to all drugs available in the market (Caracostantogolo *et al.*, 2005).

In the Mesopotamian region of Argentina, the resistance of *H. contortus*, and other species, to benzimidazoles has been reported and the increase and geographic dispersion of cases of multiple drug resistance in the central area of the country such as Santa Fe and Buenos Aires provinces are of particular concern (Anziani and Fiel, 2015).

Although new formulations appeared in the market since 2010 (i.e. monepantel and derquantel), after five years, there are already reports of resistance to monepantel in Argentina and Uruguay (Mederos, Ramos and Banchero, 2014; Cerutti *et al.*, 2018). Recently (as of September 2020) the use of the drug Naftalofos (Vermkon, König) was presented and approved in Argentina by SENASA (Servicio Nacional de Sanidad y Calidad Agroalimentaria – www.argentina.gob.ar/senasa).

The ability of parasites to generate drug resistance is certainly much faster and more efficient than expected. In this "race" for the development of new drugs and their massive and indiscriminate application, the major consequence is not only the creation of new resistant strains, but also the generation of waste that contaminates food and the environment.

There are alternative and complementary practices that aim for integrated control of parasites, such as the use of condensed tannins, the management of pastures and flocks, and a vaccine for *Haemonchus* (developed in Australia – Barvervax). Nonetheless, opportunities to apply such practices are limited to certain geographic regions and some are expensive or cannot be applied due to the type of production system in which the animals are raised. In this context, it is necessary to search for and implement new options for sheep farming in these regions that help to reduce losses, stimulate profitable and sustainable production over time, and minimize contamination with chemicals.

As reviewed by Periasamy *et al.* (2014), there is considerable variation among and within sheep breeds in their ability to resist gastrointestinal nematodes. The most widely accepted and practiced measure of resistance/susceptibility to GIN is the count of eggs per gram of faeces (FEC). This trait, in general, has a low to medium heritability (Assenza *et al.*, 2014; Vanimisetti *et al.*, 2004). The possibility of raising sheep more resistant to gastrointestinal parasites in different production systems was widely discussed, with ample evidence reported by various authors (Vagenas *et al.*, 2002; Miller *et al.*, 2006; Kemper *et al.*, 2010; Bishop, 2012; Karlsson and Greeff, 2012; Atlija *et al.*, 2016; Benavides *et al.*, 2016; Aguerre *et al.*, 2018; Stear *et al.*, 2019; Haehling *et al.*, 2020).

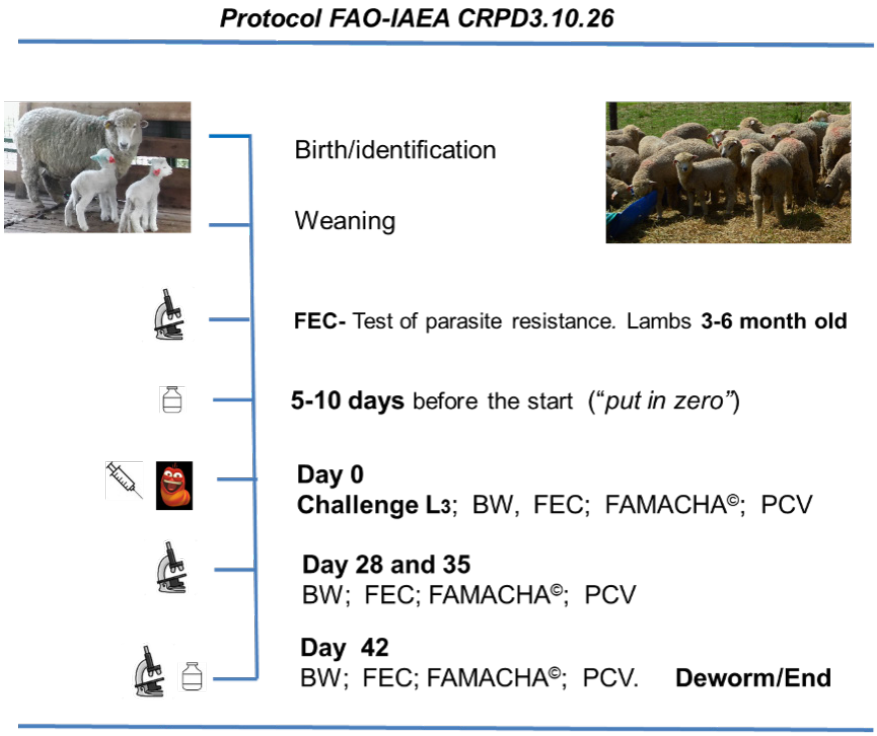
In the year 2009, the INTA´s project AERG-234002 launched a new phase of the former study on parasite resistance in sheep and the following year it was part of a multinational collaborative project sponsored by the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization of the United Nations through the IAEA Collaborative Research Project Number D3.10.26. The current study is part of this collaborative project.

The main objective of this study was to find genetic variation underlying parasite resistance in sheep to be used in breeding programmes. To accomplish that, the following specific objectives were posed: (i) to estimate variance components for body weight (BW), faecal egg count (FEC), packed cell volume (PCV), and FAMACHA© score (FAM); (ii) to estimate phenotypic and genetic correlations between these traits in Corriedale lambs after an artificial challenge with infective larvae; and (iii) to perform association analyses with a set of single nucleotide polymorphisms (SNPs) within candidate genes for immune response.

2. Materials and methods

Animals, phenotypes and genotypes

Artificial challenges were performed on 1 072 Corriedale lambs of both sexes, from 35 ram half-sib families, with complete pedigree information for three generations. The animals were born between 2010 and 2021. Procedures for challenge experiments and blood sample collection were approved by the Institutional Committee for Care and Use of Experimental Animals (CICUAE) of the National Institute of Agricultural Technology (INTA) and were carried out in strict accordance with the guidelines specified in the institutional manual. Lambs at risk were treated urgently and removed from the trials. The flocks studied belonged to two INTA experimental stations located in the northeast of Argentina. Briefly, the protocol used was as follows (Figure 1): animals were weaned 90 days after birth and kept on field until they were 4 to 6 months of age. At that time, lambs were separated by sex, dewormed, and moved to a pen. Lambs were artificially challenged via the rumen with 5 000 infective third-stage larvae (L_3) of *Haemonchus contortus*. At days 0, 28, 35 and 42 post-challenge, body weight (BW0, BW28, BW35 and BW42, respectively), faecal egg count (FEC0, FEC28, FEC35 and FEC42, respectively), packed cell volume (PCV0, PCV28, PCV35 and PCV42, respectively) and FAMACHA© (FAM0, FAM28, FAM35 and FAM42, respectively) were recorded. Later, animals were dewormed and bred in extensive systems under common flock management. The PCV was determined from total blood by the microhematocrit centrifuge method. Faffa Malan Chart (FAMACHA) score was recorded by examination of the mucous membrane of the eye and comparing it with the five-value scale of the FAMACHA© chart (Bath, Malan and Van Wyk, 1996). It was registered at each time point by the same trained technician on each location to minimize errors.



Source: Authors' own elaboration.

Figure 1: Protocol from CRPD3.10.26 FAO-IAEA

Genomic DNA was obtained from blood samples using commercial kits following the manufacturers' protocols. As a first step of the ongoing project, a total of 173 SNPs belonging to 77 candidate genes for immune response from every ovine chromosome except for chromosomes 4, 9, 10, 18, 21, 23, and Y were genotyped on 624 animals. The second step (in progress) consists in the genotyping of a larger number of animals with SNP microarrays to perform a genome-wide association study. Candidate genes and markers were selected as described in (Periasamy *et al.*, 2014). Genotyping was performed by competitive allele specific PCR (KASPar) assays based on FRET chemistry (KBiosciences, LGC Genomics, UK). Cycling conditions for each assay were those recommended by the manufacturer. BioRad CFX96 (BioRad, USA) software was utilized for genotype calling. A quality control (QC) check of the genotypes was performed using PLINK v1.9 software (Chang *et al.*, 2015). QC consisted in excluding samples with average call rate < 95 percent, as well as removing from the analysis individual SNPs with call rate < 95 percent and minor allele frequency (MAF) < 0.025. The latter parameter was determined using SNP

frequencies on founder animals to avoid bias due to inbreeding. No SNP was highly deviated from Hardy-Weinberg equilibrium (all SNPs showed *p*-values for HWE exact test > 1.10^{-6}).

Statistical analysis

FEC was not normally distributed, consequently, observed values of FEC were log transformed, LNFEC = ln (FEC + 250). Univariate analyses were conducted, using mixed model procedures in LME4 (Bates *et al.*, 2022) package in R software (R Core Team, 2014) to identify effects and covariates that contributed significantly to the variation of BW, LNFEC, PCV and FAMACHA© index. These models included, for all response variables, lamb's BW at the beginning of the trial as a covariate; farm, year of trial, and sex as fixed effects; and ram as a random effect; plus, for each response variable, its value at day zero as a covariate. Based on the selected models, univariate animal mixed models were used to estimate additive genetic variance (and estimated breeding value, EBV) for BW, LNFEC, PCV and FAMACHA© index means (days 28, 35 and 42). Phenotypic and genetic correlations were estimated using bivariate animal mixed models. Restricted maximum likelihood (REML) estimates were obtained with the EM algorithm using the WOMBAT software (Meyer, 2007).

After genotype QC, 623 lambs and 141 SNPs remained in the dataset. Association analyses between each individual SNP and the EBVs for FEC were carried out in PLINK v1.9 using linear models. Additive linear models were fitted and adjusted for significant covariate effects which included sex, farm, challenge age, PCV0, and the first two principal components derived from a principal component analysis performed using PLINK v1.9. To account for the risk of false positives due to the multiple testing problem, *p*-values were adjusted by Bonferroni correction. Corrected *p*-values < 0.05 were accepted to represent a proof of significant associations with the character under study.

3. Results and discussion

Total data reordered, structured and descriptive statistics for age (in days), body weight (BW), average faecal egg count (FEC), ln (FEC+250), package cell volume (PCV) and FAMACHA© traits in the Corriedale sheep are presented in Table 1.

Among South American countries, the most similar breeding conditions in Corriedale sheep grazing systems are those of Argentina and Uruguay. Because the protocols used in Uruguay to record descriptive traits of resistance/resilience to GIN differ in the larval challenge (natural vs artificial) and in the age of the animals, Uruguay's mean values for BW and PCV are higher (34.42 and 35.39, respectively) than in Argentina but conversely, the average values for LogFEC and FAMACHA© are smaller (6.61 and 2.53, respectively) (Ciappesoni and Goldberg, 2018).

A summary of the descriptive statistics for the phenotypic traits on Corriedale lambs at 0, 28, 35 and 42 days post-challenge is shown in Table 2.

The mean BW during the 42 days of challenge increased by 1.5 kg. There were significant differences ($p < 0.001$) between all the contrasts except between day 28 and day 35 ($p > 0.05$). Faecal egg counts increased over time, showing significant differences ($p < 0.001$) between day 0 and the rest of the days and between day 28 vs 35 and 42, and for days 35 vs 42 too. Conversely, the FAMACHA© mean increased over time from 2.7 to 3.4 with significant differences in all contrasts.

Estimated heritabilities, genetic and phenotypic correlations for BW, LNFEC, PCV and FAMACHA© means are shown in Table 3.

On average, heritabilities, genetic, and phenotypic correlations had standard errors of 0.06, 0.15 and 0.03, respectively.

The estimated h^2 were similar for FAM (0.29), PCV (0.31) and LNFEC (0.32) and higher than those reported by Balconi Marques, Goldberg and Ciappesoni (2020) for the same breed in Uruguay (0.10, 0.25 and 0.19, respectively). The h^2 for BW was 0.44 (0.05), higher than those reported by Ciappesoni and Goldberg (2018) and Balconi Marques, Goldberg and Ciappesoni (2020) (0.35 and 0.33, respectively).

The BW was favourably genetically correlated with LNFEC (-0.42) and with FAM (-0.29), however the genetic correlation with PCV was practically null (0.07). Balconi Marques, Goldberg and Ciappesoni (2020) found similar values for BW - LNFEC and FAM, but with a lower magnitude (-0.09 and -0.17).

The negative genetic correlations between PCV and FAM (-0.46) and between PCV and LNFEC (-0.65) and the positive genetic correlation found between LNFEC and FAM (0.76) are indicative of a typical response to the hematophagous parasite *Haemonchus sp*.

The negative genetic correlations between BW and LNFEC (-0.42) and the slightly positive genetic correlation between BW and PCV (0.07) are indicative of resistant and resilience traits.

The FAMACHA© score h^2 (0.29) and its positive genetic correlation with FEC (0.76) suggest it can be used as a reliable indicator of parasitism with *Haemonchus sp*.

The phenotypic and genetic negative correlations between BW and LNFEC and positive correlation with PCV allow selecting animals for both characters simultaneously with a positive response.

The minimum and maximum EBV, estimated with an accuracy of ≥ 0.7, were as follows: BW −2.4 kg to +4.2 kg; FEC -1462 to +2469; PCV +4.06 percent to −2.3 percent and FAMACHA© −0.38 to + 0.50 units.

Table 1: Descriptive statistics for age (in days) body weight (BW), average faecal egg count (FEC), Ln (FEC+250), package cell volume (PCV) and FAMACHA©

Trait	N	Mean	Standard deviation	Maximum	Minimum
Age (days)	1 072	169	20.83	237	105
Body weight (BW; kg)	1 071	23.95	4.97	40.10	11.00
Average FEC (FEC)	1 069	1 845	3 535	17 700	0.00
Ln FEC (LNFEC)	1 069	7.66	0.97	10.09	5.52
Packed cell volume (PCV)	1 062	24.68	5.05	37.50	6.67
FAMACHA© score (1-5)	1 072	3.31	0.74	5.00	1.00

Source: Authors' own elaboration.

Table 2: Descriptive statistics for the phenotypic traits during the 42 days of artificial challenge with L_3 using the CRPD3.10.26 FAO-IAEA protocol

BW Day	n	Average	SD	Max	Min
0	1 071	23.1	5.21	40.0	9.9
28	1 070	24.1	5.16	39.9	10.6
35	1 063	24.2	5.04	43.0	11.3
42	1 059	24.6	5.29	40.6	10.5
FEC Day	**n**	**Average**	**SD**	**Max**	**Min**
0	1 069	0	0.00	0	0
28	1 050	2 460	3 296.85	38 300	0
35	1 045	3 644	4 163.20	28 400	0
42	1 056	4 599	6 227.50	58 700	0
PCV Day	**n**	**Average**	**SD**	**Max**	**Min**
0	1 062	27.8	4.63	42	9
28	1 056	25.2	4.99	38	7
35	920	24.7	5.45	42	7
42	1 047	24.2	5.52	38	5
FAM Day	**n**	**Average**	**SD**	**Max**	**Min**
0	1 072	2.7	1.06	5	1
28	1 068	3.1	0.92	5	1
35	1 061	3.3	0.85	5	1
42	1 056	3.4	1.07	5	1

Source: Authors' own elaboration.

Table 3: Heritabilities, genetic and phenotypic correlations, and their standard deviations

Trait	BW	FAM	PCV	LNFEC
BW	**0.44** (0.05)	-0.29 (0.13)	0.07 (0.15)	-0.42 (0.12)
FAM	-0.36 (0.03)	**0.29** (0.06)	-0.46 (0.12)	0.76 (0.10)
PCV	0.20 (0.04)	-0.50 (0.03)	**0.31** (0.07)	-0.65 (0.12)
LNFEC	-0.29 (0.05)	0.42 (0.03)	-0.50 (0.03)	**0.32** (0.06)

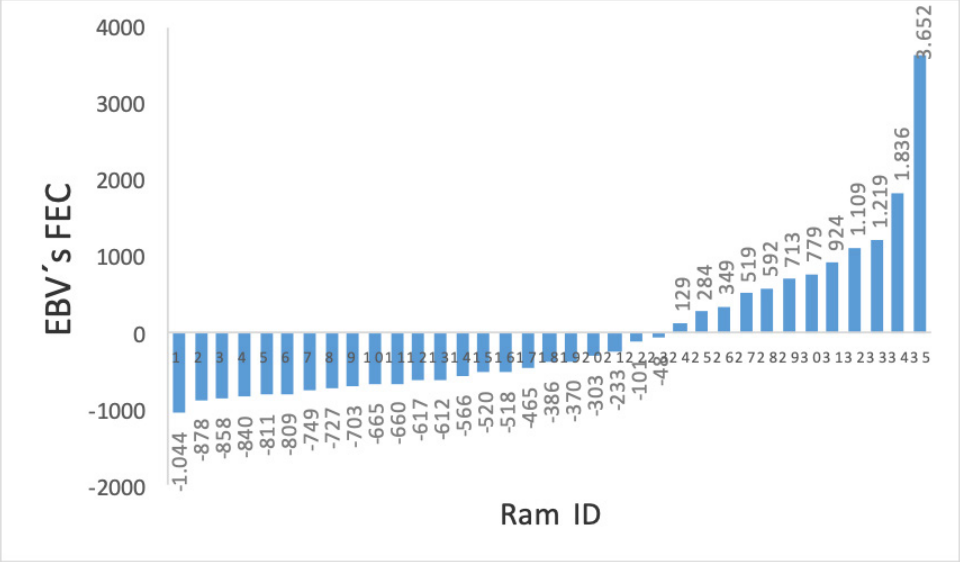
Source: Authors' own elaboration.

Note: Heritabilities on the diagonal, genetic correlations above the diagonal and phenotypic correlations below the diagonal. Standard deviations between brackets.

Figure 2 shows the distribution of FEC EBVs for the 35 Corriedale rams with at least 10 progeny. These EBVs are relative to the mean of FECs after back-transforming LNFEC; in 2020, that mean was equal to 3092. The maximum and minimum FEC EBVs values observed in rams were 3 652 and -1 044 respectively. EBVs are indicators of the genetic merit of each ram and can be used to predict future changes for FEC of their progenies (EPDs).

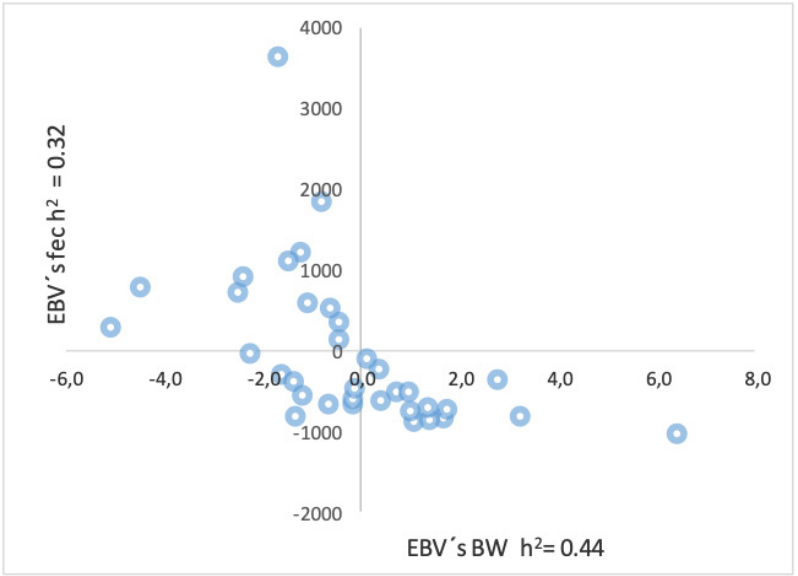
Figure 3 shows the distributions of FEC and BW EBVs for the 35 rams. We found that 37 percent (13/35) of the rams had negative values for FEC EBV and positive values for BW EBV.

Seven SNPs showed significant corrected *p*-values < 0.05 when testing association to FEC EBVs (Table 4). Among those significant associated markers, we found that OLADRA1_479, a SNP in the MHC-Ovine Lymphocyte Antigen DRA gene on OAR20, showed the lowest *p*-value. Then we found four SNPs on OAR3 (in decreasing order of significance: CLEC12A_567, CLEC8A_532, CLEC12A_440, and IL2RB_180). Those SNPs were located in two genes of C-type lectin domain families and in the Interleukin 2 receptor β gene. The last two SNPs associated with FEC EBVs obtained in this study and adapted from Raschia *et al.* (2021) were TLR10_292 and MASP2_104, on sheep chromosomes 6 and 12, respectively, which corresponded to the Toll-like receptor 10 and the Mannan binding lectin serine peptidase 2 genes (Table 4).



Source: Authors' own elaboration.

Figure 2: FEC EBVs distribution for 35 Corriedale rams



Source: Authors' own elaboration.

Figure 3: BW and FEC EBVs distribution for 35 Corriedale rams

Table 4: SNPs associated to FEC EBVs

SNP	Position (OAR:bp) [1]	Candidate gene	Bonferroni-corrected *p*-value
OLADRA1_479	20:25775019	*OLA-DRA*	9.21 e-7
CLEC12A_567	3:204592787	*CLEC12A*	0.001683
CLEC8A_532	3:204456657	*CLEC8A*	0.001752
CLEC12A_440	3:204592660	*CLEC12A*	0.002115
IL2RB_180	3:180362559	*IL2RB*	0.002177
TLR10_292	6:57993319	*TLR10*	0.002937
MASP2_104	12:40735932	*MASP2*	0.01938

Source: Authors' own elaboration.

Significant SNPs found to be associated with FEC EBV are located in genes involved in different stages of the pathogen-host interaction, such as pathogen recognition (*TLR* and C-type lectin domain gene families), innate immune response (*MASP* and *TLR*), and adaptive response to infection (C-type lectin domain gene families, *IL2RB*, and *OLA-DRA*).

4. Final remarks

Gastrointestinal nematodes in sheep have rapidly become resistant to all drugs developed in recent years and there is no doubt about the need for integrated management strategies for the control of parasites. In addition to pasture rotation, deworming strategies involving FAMACHA© score, alternative antiparasitics (i.e. tannins), among other practices, the use of genetically more resistant and resilient sheep is envisaged as the most sustainable and "clean" strategy over time.

The CRPD3.10.26 FAO-IAEA protocol used for more than 10 years allowed us not only to obtain phenotypic and genotypic information for a breeding programme in the Corriedale breed by having the components of variance, correlations and heritabilities, but also to find the genetic variability underlying GIN resistance and resilience traits in sheep. Furthermore, none of the challenged animals was negatively affected neither for growth nor for health and the artificial challenge with L_3 allowed us in a short time, 35–40 days, to obtain data on resistance and resilience to GIN.

The results indicate that the Corriedale breed has sufficient genetic variability for the four traits studied and the values of heritability and genetic and phenotypic correlations obtained support a potential use for genetic progress in all traits.

The high genetic correlation between FEC and FAMACHA© estimated in this work (0.76) and the negative genetic correlation between FEC and PCV (-0.46) suggest the possibility of using both traits to select animals for resistance and resilience to GIN.

The protocol presented in this study requires repeated measures, however. An alternative could be to do only one FEC and FAMACHA© score determination at day 35. The correlation found between the average of FEC28, FEC35, and FEC42 and FEC on day 35 was 0.93 in this study.

Currently, the protocol is being used in four experimental units and in two private stud flocks in Corriedale, Texel and Ideal breeds.

By means of the association analyses performed using the candidate gene approach, we identified single SNPs that had a significant association with nematode resistance in Corriedale sheep. This information has potential use in marker-assisted breeding schemes of Corriedale sheep in Argentina, which constitute a promising long-term strategy to effectively reduce parasitic infections and enable a profitable and sustainable sheep production. Nonetheless, we should further explore the variability in loci not included in this study to have a clearer picture of the genetic regions underlying GIN resistance in sheep.

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