ANIMAL GENETICS AND GENOMICS

Comparison between methods for measuring fecal egg count and estimating genetic parameters for gastrointestinal parasite resistance traits in sheep

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Abstract

Fecal egg count (**FEC**) is an indicative measurement for parasite infection in sheep. Different FEC methods may show inconsistent results. Not accounting for inconsistencies can be problematic when integrating measurements from different FEC methods for genetic evaluation. The objectives of this study were to evaluate the difference in means and variances between two fecal egg counting methods used in sheep—the Modified McMaster (**LMMR**) and the Triple Chamber McMaster (**LTCM**); to estimate variance components for the two FEC methods, treating them as two different traits; and to integrate FEC data from the two different methods and estimate genetic parameters for FEC and other gastrointestinal parasite resistance traits. Fecal samples were collected from a commercial Rideau-Arcott sheep farm in Ontario. Fecal egg counting was performed using both LMMR and the LTCM methods. Other parasite resistance trait records were collected from the same farm including eye score (**FAMACHA**), body condition score (**BCS**), and body weight (**WT**). The two FEC methods were highly genetically (0.94) and phenotypically (0.88) correlated. However, the mean and variance between the two FEC methods were significantly different ($P < 0.0001$). Therefore, re-scaling is required prior to integrating data from the different methods. For the multiple trait analysis, data from the two fecal egg counting methods were integrated (**LFEC**) by using records for the LMMR when available and replacing missing records with re-standardized LTCM records converted to the same mean and variance of LMMR. Heritability estimates were 0.12 ± 0.04, 0.07 ± 0.05, 0.17 ± 0.06, and 0.24 ± 0.07 for LFEC egg count, FAMACHA, BCS, and WT, respectively. The estimated genetic correlations between FEC and the other parasite resistance traits were low and not significant ($P > 0.05$) for FAMACHA ($r = 0.24 \pm 0.32$) and WT ($r = 0.22 \pm 0.19$), and essentially zero for BCS ($r = -0.03 \pm 0.25$), suggesting little to no benefit of using such traits as indicators for LFEC.

Key words: FAMACHA, fecal egg count, fecal egg counting methods, gastrointestinal parasite resistance, sheep

Introduction

Haemonchus contortus is a parasitic gastrointestinal nematode (**GIN**) that infects grazing sheep. High levels of *H. contortus* infection can cause economic losses for producers as the result of increased morbidity or mortality of sheep. Frequent or improper drug administration to treat *H. contortus* infection have contributed to the evolution of drug resistance in most parasite populations, rendering these treatments ineffective

Abbreviations

BCS	body condition score
FEC	fecal egg count
GIN	gastrointestinal nematode

(Emery et al., 2016). Thus, alternate methods for combating *H. contortus* infection are needed for sheep producers to keep their flocks healthy and productive. There is evidence that some populations of sheep are genetically resistant to GIN infection (Baker, 1998), so resistance to GIN has become a target for selective breeding decisions. Such selected sheep populations will become resistant to GIN infections over time and drug administration would no longer be necessary or will be minimized. Fecal egg count (**FEC**) is a well-used tool in the diagnosis of GIN infection and is usually considered a quantitative measure for the level of infection in sheep. However, there are some limitations for using FEC as an indicator of the parasite burden of the host, which are often overlooked, such as the fact that FEC is a ratio (eggs per gram of feces) in which any factor that changes the volume of faces (e.g., dry matter, food quality, etc.) can affect the measure (Greer and Sykes, 2012). In mixed infections, the interpretation of FEC should consider the female fecundity of the considered species, for instance, female *H. contortus* are able to lay thousands of eggs per day in comparison to *Trichostrongilus* spp., which only produces few hundred (Coyne et al., 1991). In addition, there are resilient animals showing high/moderate FEC values, for which the production performance is not compromised. In this case, FEC would not be a direct measure of the cost of the infection for the host (Greer and Sykes, 2012). Nevertheless, FEC has been widely used as an indicator for host resistance to GIN in sheep showing to be a heritable trait, with a moderate heritability of 0.27 as reviewed by Safari et al. (2005). Therefore, genetic improvement for parasite resistance is possible by using FEC as a phenotype for genetic selection.

There are many methods used to measure FEC in sheep (e.g., the Modified McMaster method and the Triple Chamber McMaster method, among others) that vary in terms of sample weight, floatation solution, centrifugation, number of McMaster chambers, and the precision of egg detection (e.g., Cebra and Stang, 2008; Vadlejch et al., 2011; Paras et al., 2018). The Modified McMaster method is the most common method used to quantify the GIN infection in sheep, which uses a two-chamber slide to count the eggs per gram and can detect as low as 50 eggs/g. However, this methodology has been shown to have lower sensitivity than Mini-FLOTAV for FEC lower than <500 eggs/g (Amadesi et al., 2020). On the other hand, the Triple Chamber McMaster method uses three-chamber slide to count the eggs per gram and can detect as low as 8 eggs/g. Thus, the major difference in the two FEC method is the number of chambers and the detection limit. Variation in FEC methodology may lead to different estimates of parasite load, even within the same sample. Thus, assessment of FEC trends over time or across populations becomes difficult or impossible if these differences in method are not accounted for. In FECs conducted for new world camelid species, Cebra and Stang (2008) compared six quantitative methods: centrifugation-sucrose flotation after 10 min, centrifugation-sucrose flotation after 60 min, saturated saline McMaster for 15 min, saturated saline McMaster for 60 min, sucrose McMaster for 15 min, and sucrose McMaster for 60 min. Their results indicated that some methods provide similar observations for eggs/g, while others were different by factor of up to 8-fold. Paras et al. (2018) compared three fecal egg counting methods: the

Modified-Wisconsin, the 3-chamber McMaster, and the Mini-FLOTAC in sheep, cattle, horses, and llamas. They reported an increase in number of observed eggs in sheep by 116.5%, 223.3%, and 49.4% when comparing Mini-FLOTAC to McMaster, Mini-FLOTAC to Wisconsin, and McMaster to Wisconsin, respectively. Cringoli et al. (2004) reported significant influence of the type of flotation solution, sample dilution, and McMaster counting area on the observations for FEC in sheep for both gastrointestinal strongyles and *Dicrocoelium dendriticum*. The consistency for phenotypic measurements is essential for successful genetic evaluation, thus it is important records to be consistent and comparable across populations.

In addition to FEC, there are alternative measurements that can be used to indicate the level of parasite infection in small ruminants such as FAMACHA eye score and body condition score (**BCS**). The FAMACHA eye score is a method that assesses the tint of redness of the mucus membrane of the animals' eye, which indicates animal's state of anemia on a scale of 1 to 5 (presumed to be proportional to the level of GIN infection; Kaplan et al., 2004; Reynecke et al., 2011). The BCS is a measurement of the fat and the flesh covering the back and the loin of the animals. Animals are given a score from 1 to 5 indicating their level of body condition. Animals in good body condition are presumed to be under little stress from GIN infection. In addition, previous studies indicate that such traits were genetically associated with parasite resistance with low to moderate heritabilities, ranged between 0.08 and 0.49, suggesting that they would be useful for selective breeding decisions (Riley and van Wyk, 2009; Cloete et al., 2016; Álvarez et al., 2018; Snyman and Fisher, 2019).

The FAMACHA and BCS traits offer a clear advantage to farmers compared with FEC as they can be conducted on-farm and do not require specific laboratorial equipment. Thus, these traits could be an attractive alternative for producers to be used as phenotypes for genetic selection for parasite resistance.

The overall objective of this study was to estimate genetic parameters for FEC using data from two different laboratory methodologies for FEC detection, the "modified McMaster" and the "triple chamber McMaster," including genetic correlations with other parasite resistance traits, which were FAMACHA, BCS, and body weight (**WT**). The specific steps carried out to achieve this overall objective were to: 1) evaluate the differences in mean and variance between the FEC records using two different methods: the "Modified McMaster" and the "Triple Chamber McMaster"; 2) estimate the variance components for the two FEC methods, treating them as two different traits; and 3) integrate FEC data from the two different methods and estimate the genetic parameters, including genetic correlation with other parasite resistance traits, that is, FAMACHA, BCS, and WT.

Materials and Methods

Data used in this study was provided by a commercial sheep farm located in Ontario based on routine recordings of animals, which followed the 2013 National Farm Animal Care Council's Code of Practice for the Care and Handling of Sheep (https://www.nfacc.ca/sheep-code). Therefore, no approval was required for this study from the Institutional Animal Care and Use Committee (IACUC) or its equivalent.

Collection of fecal samples and phenotypic records

Fecal samples (between the years 2012 and 2019) and phenotypic records (between the years 2016 and 2019) for WT, BCS, and FAMACHA were collected during the grazing season (from May to

October) from pure-bred Rideau-Arcott sheep at breeding age from a commercial sheep farm in Ontario, Canada. A summary of the pedigree structure of the flock is shown in Table 2. The rams and ewes were managed separately during the grazing season each year. Hence, the management group definition was based on sex and year. Animals were sampled and recorded at different dates of the grazing season, with some repeated records for the same animals. The date of measurement and the management group were later combined to form a group of measurement. The FAMACHA score was taken by assessing the redness of the mucus membrane of the eyes, indicating the anemic level from scale between 1 (red and normal) and 5 (white and severely anemic). The BCS was taken by assessing the fat and flesh covering the back and the loin of each animal with scores ranging between 1 (extremely thin) and 5 (extremely fat). Fecal samples were collected from the rectum of each animal and stored using sealable plastic bags. Samples were immediately placed in a cooler with ice packs to be transported to the laboratories, where they were refrigerated at 4 °C to be analyzed the next day. The FEC was performed on the fecal samples using two different methods: 1) the Modified McMaster (MMR), with lower detection limit of 50 eggs/g and 2) the Triple Chamber McMaster (TCM), with lower detection limit of 8 eggs/g.

Between the years 2012 and 2019, fecal samples were sent to the Animal Health Lab at the Ontario Veterinary College to perform fecal egg counting using the Modified McMaster method. The method was described by Zajac et al. (2012). Following Zajac et al. (2012) protocol, fecal sample of 4 g were mixed well with 56 mL of floatation solution. The mixture was then strained and put in the two-chamber McMaster slide and allowed to sit for 5 min before counting the eggs under microscope.

In most recent years (2018 and 2019), additional fecal egg counting was performed at the Department of Animal Biosciences at the University of Guelph using the Triple Chamber McMaster method, in which a 4-g fecal sample is mixed and homogenized with 26 mL of floating solution (300 g L^{-1} NaCl). The mixture is then strained and put into Triple Chamber McMaster slides and left to sit no less than 5 min before the initiation of egg counting under the microscope.

Records with zero fecal eggs count were adjusted to half of the lower detection limit to account for differences in FEC methods. Therefore, the minimum value for FEC adjusted observations were 25 and 4 eggs/g for the MMR and TCM methods, respectively. These adjusted minimum values took into account the sensitivity of each of the two methods used to determine FEC, that is, 50 eggs/g for MMR (Pereckiene et al., 2007) and 8 eggs/g for TCM (Westers et al., 2016). FEC values from both methods were transformed by taking the natural logarithm (ln) of egg counts (**LMMR** and **LTCM**, respectively) in an attempt to normalize their distributions for the analyses. The total number of records for each trait phenotype and their basic descriptive statistics are presented in Table 1.

Evaluation and comparison between different methods to measure FEC

Whenever the phenotypic records were available for both FEC methods, the differences were evaluated at each year using paired t-test. The number of ln FEC records available for both methods in the years 2018 and 2019 were 115 and 87, respectively, with 202 records available in total. In addition, using all records, the means were compared using Welch t-test, and the homogeneity of variance for the ln FEC records from the two different FEC methods was tested using Levens' test. All statistical tests were performed using R (R Core Team, 2015).

Estimating genetic parameters for the two FEC methods as two separate traits

The variance components for LMMR and LTCM were estimated by restricted maximum likelihood using ASREML (Gilmour et al., 2015) using a bivariate model, as follow:

$$y_{tijklm} = Y_{ti} + M_{tj} + a_{tk} + pe_{tk} + C_{tl} + e_{tijklm},$$

where y_{tijklm} is the phenotypic record for trait t; Y_{ti} is the fixed effect of the year; M_{tj} is the fixed effect of the month; a_{tk} is the random animal additive genetic effect; pe_{tk} is the random animal permanent environmental effect; C_{tl} is the random group measurement effect, which was formed on based on management group (year-sex) and the date of measurement; and e_{tijklm} is the random residual error.

The variance and covariance matrix for the bivariate analysis was:

$$\begin{bmatrix} A\sigma_{a1}^2 & A\sigma_{a12} & 0 & 0 & 0 & 0 & 0 & 0 \\ A\sigma_{a21} & A\sigma_{a2}^2 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & I\sigma_{pe1}^2 & I\sigma_{pe12} & 0 & 0 & 0 & 0 \\ 0 & 0 & I\sigma_{pe21} & I\sigma_{pe2}^2 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & I\sigma_{c1}^2 & I\sigma_{c12} & 0 & 0 \\ 0 & 0 & 0 & 0 & I\sigma_{c21} & I\sigma_{c2}^2 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e1}^2 & I\sigma_{e12} \\ 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e21} & I\sigma_{e2}^2 \end{bmatrix},$$

Table 1. Basic descriptive statistics for the studied parasite resistance traits

Trait	No. of records	Range	Mean ± SD	CV (%)
LMMR	998	3.22–9.77	5.86 ± 1.54	26.34
LTCM	678	1.39–9.66	4.34 ± 2.14	49.26
LFEC	1,474	3.22–9.77	5.79 ± 1.55	26.80
FAMACHA	1,048	1–5	2.70 ± 0.78	28.96
BCS	1,054	1–5	2.83 ± 0.62	21.96
WT	1,103	23.00–96.00	56.23 ± 12.17	21.65
MMR	998	25–17,500	959.44 ± 1,699.65	177.15
TCM	678	4–15,644	581.87 ± 1,681.76	289.03
FEC	1,474	25–17,500	959.40 ± 1,758.96	183.34

LMMR, ln fecal egg count using the Modified McMaster method; LTCM, ln fecal egg count using the triple Chamber McMaster method; LFEC, integrated ln fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA, eye score system for red tint level of the color of the mucus membrane of the eyes, ranging from red "1" to "5" pale; BCS, body condition score; WT, body weight (kg). MMR, TCM, and FEC, original fecal counts before the ln transformation for LMMR, LTCM, and LFEC, respectively.

where the variances are on the diagonal, and the covariances are off of the diagonal; the subscripts a, pe, c, and e indicate the additive genetic, permanent environmental, group measurement, and residual effects, respectively, and subscripts 1 and 2 refers to the traits; A is the numerator additive relationship matrix; and I is an identity matrix.

The repeatability for FEC methods was calculated as follows:

$$r = \frac{\sigma_a^2 + \sigma_{pe}^2}{\sigma_a^2 + \sigma_{pe}^2 + \sigma_c^2 + \sigma_e^2},$$

where r is the repeatability for the trait and σ_a^2, σ_{pe}^2, σ_c^2, and σ_e^2 are the additive genetic, permanent environmental, group of measurement, and residual effect variances, respectively.

Integrating FEC data from different methods

Whenever records using the LMMR methods were available, they were used. Missing values for LMMR were replaced by adjusted LTCM by shifting and scaling the observations to the LMMR records' as follows (for mean and variance, see Table 1):

$$LTCM_i^* = \left(\frac{LTCM_i - \overline{LTCM}}{s_{LTCM}} \right) s_{LMMR} + \overline{LMMR},$$

where $LTCM_i^*$ is the re-scaled ln fecal egg count record i; $LTCM_i$ is ln fecal egg count record using TCM method; $\overline{LTCM}$ and $\overline{LMMR}$ are the means for ln fecal egg count using TCM and MMR, respectively; and s_{LTCM} and s_{LMMR} are the standard deviations for ln fecal egg count records using TCM and MMR, respectively.

The integrated fecal egg count data (LMMR + LTCM*) will be called LFEC hereafter in this article.

Estimating genetic parameters among parasite resistance traits

The covariance components were estimated by restricted maximum likelihood using ASREML (Gilmour et al., 2015), using 4-trait model for LFEC, FAMACHA, BCS, and WT, as follow:

$$y_{tijklm} = Y_{ti} + M_{tj} + a_{tk} + pe_{tk} + C_{tl} + e_{tijklm},$$

where y_{tijklm} is the phenotypic record for trait t; Y_{ti} is the fixed year effect; M_{tj} is the fixed month effect; a_{tk} is the random animal additive genetic effect; pe_{tk} is the random animal permanent environmental effect; C_{tl} is the random group of measurement effect, which was formed on based on management group (year-sex) and date of measurement; and e_{tijklm} is the random residual error.

The variance and covariance matrix for the multivariate analysis was:

$$
\begin{bmatrix}
A\sigma_{a1}^2 & \cdots & A\sigma_{a14} & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
\vdots & \ddots & \vdots & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
A\sigma_{a41} & \cdots & A\sigma_{a4}^2 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & I\sigma_{pe1}^2 & \cdots & I\sigma_{pe14} & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & \vdots & \ddots & \vdots & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & I\sigma_{pe41} & \cdots & I\sigma_{pe4}^2 & 0 & 0 & 0 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{c1}^2 & \cdots & I\sigma_{c14} & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & \vdots & \ddots & \vdots & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{c41} & \cdots & I\sigma_{c4}^2 & 0 & 0 & 0 \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e1}^2 & \cdots & I\sigma_{e14} \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & \vdots & \ddots & \vdots \\
0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & I\sigma_{e41} & \cdots & I\sigma_{e4}^2
\end{bmatrix},
$$

where the variances are on the diagonal, and the covariances are off of the diagonal; the subscripts a, pe, c, and e indicate the additive genetic, permanent environmental, group of measurement, and residual effects, respectively, and subscripts 1 to 4 refers to the traits; A is the numerator additive relationship matrix; and I is an identity matrix.

Results and Discussion

Descriptive statistics

The basic descriptive statistics for LMMR, LTCM, LFEC, FAMACHA, BCS, and WT are shown in Table 1. Figure 1 shows the number of phenotypic records for the traits over the different years. LMMR and LTCM are measures of the same phenotype (i.e., ln fecal egg count) using different methods. The mean LMMR (mean = 5.86 ln egg/g) was greater than mean LTCM (mean = 4.37 ln egg/g), while the standard deviation for LMMR (SD = 1.54 ln egg/g) was smaller than LTCM (SD = 2.14 ln egg/g). This results in greater coefficient of variation for LTCM compared with LMMR, 49.26% and 26.34%, respectively. The LFEC is the integrated ln fecal egg count from both LMMR and LTCM methods, prioritizing the LMMR records over LTCM. Whenever LMMR records were missing, they were replaced by the re-scaled LTCM records.

Differences between FEC observation using two different methods

Results of the comparisons between LMMR and LTCM using a paired t-test are presented in Table 3. In 2018, the mean difference between the two FEC methods was 0.87, significantly different from zero ($P < 0.0001$). In 2019, the mean difference between the measurements was 0.08 and not significantly different from zero ($P = 0.3858$). Thus, the results were not consistent across the 2 years. This is because the two methods are different in their lower detection limit, causing large disparities between measurements in animals with low infection levels. Figure 2 shows a scatter plot for the two FEC methods (LMMR against LTCM) for FEC data from the 2 years. Figure 3 presents a scatter plot of the difference between methods relative to the observation against the infection level as measured by LTCM (i.e., |LMMR – LTCM|/LTCM against LTCM) for FEC data from the 2 years. The association between the two FEC methods is positive and increases as the infection levels increases (Figure 2). The relative absolute difference between the two FEC methods decreases rapidly with the increase of infection levels from LTCM = 1 to 4 (low infection levels), then it decreases gradually, approaching zero in high infection levels (LTCM ~ 10; Figure 3). Indeed, the infection levels in the year 2019 were higher than 2018, which can be seen in Figures 2 and 3 and in their corresponding supplementary figures of untransformed data (Supplementary Figures S1 and S2, respectively). According to Cringoli et al. (2004), the type of the flotation solution significantly influenced the observation for FEC in sheep for both gastrointestinal strongyles and *D. dendriticum*. Different egg recovery rates were also reported when using different McMaster methods for detecting *Teladorsagia circumcincta*, including with differences due to sample weight, floatation solution, centrifugation, number of McMaster chambers, and multiplication factor (Vadlejch et al., 2011). Paras et al. (2018) compared three different fecal egg counting methods: the Modified-Wisconsin, the 3-chamber McMaster, and the Mini-FLOTAC in different host species including in sheep, cattle, horses, llamas, and spiked sample. When comparing Mini-FLOTAC to McMaster, they found
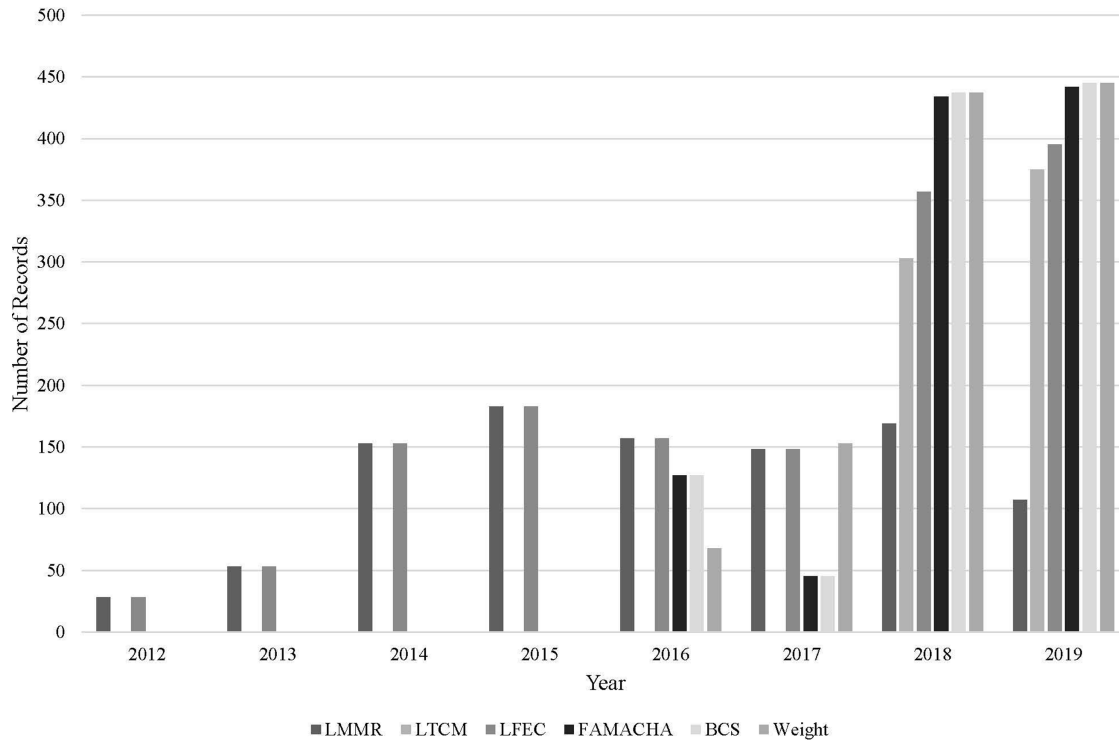
Figure 1. Distribution of number of phenotypic records per year.

Table 2. Descriptive statistics of the pedigree used in the analyses

Longest ancestral path	Number of occurrences
0	35
1	18
2	17
3	26
4	56
5	66
6	53
7	77
8	86
9	107
10	231
11	265
12	213
13	126
Total no. of individuals	1,376
No. of sires	94
No. of dams	561
No. of founders	35
Average number of discrete generation	5.16

Table 3. Comparison of the two fecal egg counting methods (LMMR and LTCM) between two different years of recording[1]

Year	n	Mean difference (95% CI)	P-value
2018	115	0.87 (0.71 to 1.03)	<0.0001
2019	87	0.08 (−0.10 to 0.25)	0.3858
Both years	202	0.52 (0.40 to 0.65)	<0.0001

[1]LMMR, fecal egg count using the Modified McMaster method in natural logarithm scale; LTCM, fecal egg count using the Triple Chamber McMaster method in natural logarithm scale.

the percent increase in number of eggs observed of 166.5%, 27.2% to 53.6%, 4.8%, 1.5%, and 28.8%, in sheep, cattle, horse, llama, and spiked sample, respectively. Also, when comparing Mini-FLOTAC to Wisconsin, they found the percent increase in number of eggs observed of 223.3%, 116.5% to 139.8%, 102.6%, 129.0%, and 130.9%, in sheep, cattle, horse, llama, and spiked sample, respectively. When comparing McMaster to Wisconsin, they found the percent increase in number of eggs observed of 49.4%, 40.9% to 88.5%, 93.4%, 98.8%, and 79.3%, in sheep, cattle, horse, llama, and spiked sample, respectively. Therefore,

inconsistencies among method used for fecal egg counting can be problematic for genetic evaluation, especially when such inconsistencies are not accounted for. This reflects a potential practical problem for commercial sheep producers, where consistency may be difficult to maintain. Thus, information about the FEC method used has important considerations in the integration of data for genetic evaluation.

Results for testing the equality of mean and variance between LMMR and LTCM are shown in Table 4. Both means and variances were significantly different ($P < 0.0001$). Therefore, the integrating records from the two FEC methods should be carefully done. It must be noted that the "Triple Chamber McMaster" method is more sensitive than the "Modified McMaster." However, when FEC data were integrated (LFEC), the LMMR records were preferred over LTCM when both were available for an animal. This was done because the majority of the FEC data were performed using the LMMR (see Table 1).

Genetic parameters for the two FEC methods as two different traits

The genetic parameters estimate from bivariate analysis treating ln FEC from different methods as two separate traits
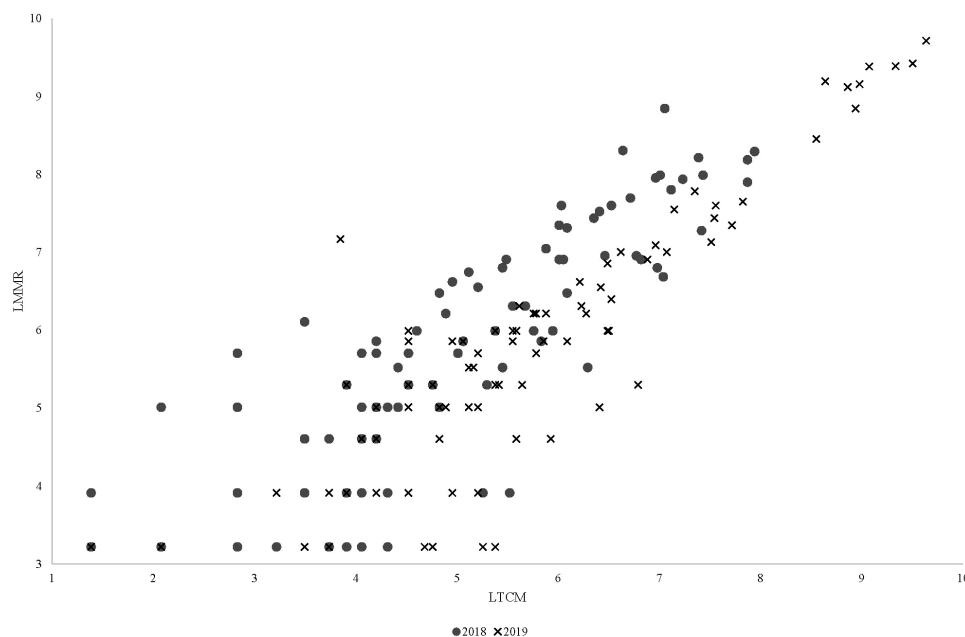
Figure 2. Scatter plot of LMMR values against LTCM values for the recording years 2018 and 2019.

(LMMR and LTCM) are given in Table 5. The estimated heritability for both methods was low, 0.10 (± 0.04) and 0.07 (± 0.05) for LMMR and LTCM, respectively. The heritability estimate for FEC ranges between 0.00 and 0.37 (Snyman and Fisher, 2019) and averaged 0.27 in Safari et al. (2005). Heritability estimates reported in this study were similar to those in Burkina Faso and South Africa, which range from 0.06 to 0.17 (Álvarez et al., 2018), and 0.06 to 0.16 (Matebesi-Ranthimo et al., 2014; Mpetile et al., 2015; Cloete et al., 2016), respectively.

The repeatability estimates for LMMR and LTCM were 0.18 and 0.25, respectively. The expected heritability for the mean of n records (h_n^2) can be calculated by $h_n^2 = n \times h^2/[1 + (n-1) \times r]$ (Falconer and Mackay, 1996), where n is the number of records; h^2 is the estimated heritability for a single record; and r is the estimated repeatability. The expected heritabilities for the mean of three records for LMMR and LTCM were 0.22 and 0.14, respectively, which increased by factor of 2.2 and 2.0 relative to one record, respectively. Therefore, LMMR method benefits more from repeated records than LTCM method. In the current data, the average number of records per animal was 1.30 and 1.80 for LMMR and LTCM, respectively.

In this study, estimates for correlations between LMMR and LTCM (Table 5) were very high for genetic (r_g = 0.94), group measurement (r_c = 0.99), and permanent environmental effect (r_{pe} = 0.93), and high for residual (r_e = 0.82) effect, leading to high phenotypic correlation (r_{ph} = 0.88). This was expected since both LMMR and LTCM try to measure the same phenotype. These results support the integration of these tow FEC measures into a single measure as done in this study (i.e., LFEC).

Estimating genetic parameters for all parasite resistance traits

Estimates of genetic parameters from multi-trait analysis for parasite resistance traits (including LFEC, FAMACHA, BCS, and WT) are shown in Table 6. The heritability estimates were low, ranging from 0.07 to 0.24 (Table 6). The heritability estimate for

LFEC was 0.12 (±0.04), which was slightly higher than estimates for LMMR and LTCM from the bivariate analysis.

The heritability estimate for FAMACHA 0.07 (±0.05) was low with a large standard error (Table 6). Snyman and Fisher (2019) reported heritability estimates for FAMACHA from 0.08 to 0.49. Riley and van Wyk (2011) reported similar heritability estimates in South African Merinos under moderate GIN challenge (0.08 ± 0.04) and higher heritability in severe GIN challenge (0.17 ± 0.05). In the same study, different heritability estimates were reported depending on adjustment methods made to the treated animals under moderate and severe GIN challenges (range from 0.07 to 0.16, and 0.17 to 0.23, respectively). In another study of the same South African Merino population, Riley and van Wyk (2009) reported heritability of 0.13 in low GIN challenge and heritability estimates ranging from 0.08 to 0.11 and from 0.20 to 0.24 in moderate and severe GIN challenges, respectively. Cloete et al (2016) reported heritability of 0.13 in Mediterranean Region of South Africa. In Burkina Faso, heritability reported ranged from 0.21 to 0.34 (Álvarez et al., 2018). Higher heritability estimates for FAMACHA score (0.33 to 0.41) were reported in Dorper Sheep in South Africa (Ngere et al., 2017). The sheep in this study did not have large parasite loads and did not show disease symptoms. This is likely the main reason why the heritability estimate was similar to the studies with moderate GIN challenge.

In this study, the heritability estimated for BCS was 0.17 ± 0.05 (Table 6). Riley and van Wyk (2009) reported heritability of 0.17, 0.26 to 27, and 0.29 to 0.33, in low, moderate, and severe GIN challenges, respectively. The heritability estimate (h^2 = 0.24 ± 0.07) of WT was the highest in this study (Table 6). Riley and van Wyk (2009) reported heritability of 0.19, 0.30 to 35, and 0.29 to 0.32, in low, moderate, and severe GIN challenges, respectively, for WT of lambs of similar age to the ones used in the present study.

The estimates for genetic and phenotypic correlations, are presented in Table 7. The genetic correlation between LFEC and
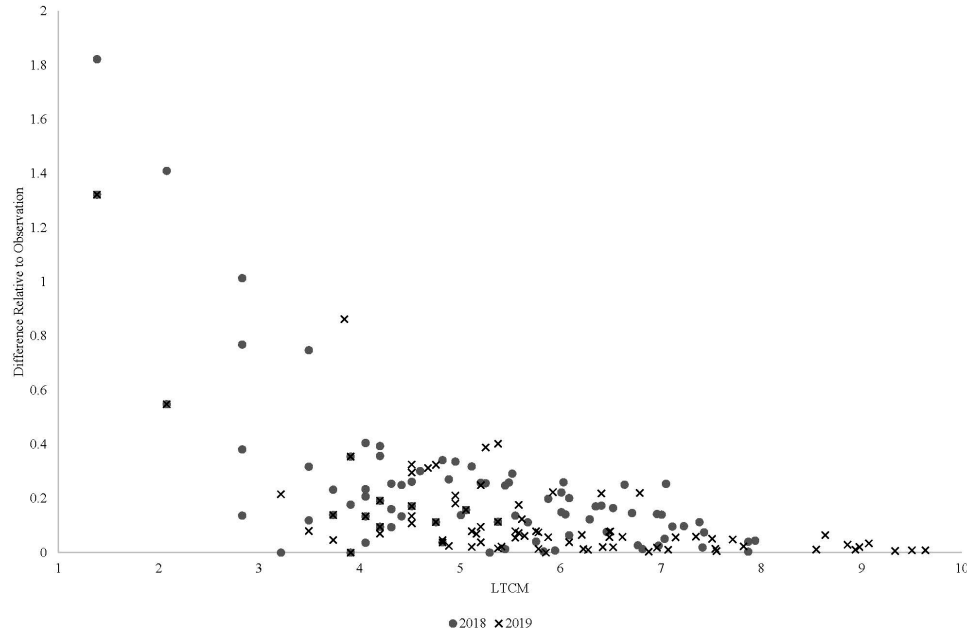
Figure 3. Scatter plot of the absolute difference between FEC methods divided by LTCM (i.e., |LMMR – LTCM|/LTCM) against LTCM for the recording years 2018 and 2019.

Table 4. Test for equality of variance (Levens' test) and mean (Welch t-test) for alternate fecal egg counting method

Groups compared		Levens' test		Welch t-test		
Method[1]	Mean ± SD	F	P-value	t	df	P-value
LMMR	5.86 ± 1.54	118.98	<0.0001	15.864	1,143.2	<0.0001
LTCM	4.34 ± 2.137					

[1]LMMR, ln fecal egg count using the Modified McMaster method; LTCM, ln fecal egg count using the triple Chamber McMaster method.

Table 5. Estimates for genetic parameters (±SE) for the two fecal egg counting methods (LMMR and LTCM)

	Trait[1]	
Estimate[2]	LMMR	LTCM
σ_a^2	0.30 ± 0.11	0.25 ± 0.17
σ_c^2	0.90 ± 0.29	1.09 ± 0.36
σ_{pe}^2	0.22 ± 0.12	0.64 ± 0.18
σ_e^2	1.43 ± 0.11	1.65 ± 0.12
h^2	0.10 ± 0.04	0.07 ± 0.05
r^2	0.18 ± 0.04	0.25 ± 0.04
	Correlation	
r_g	0.94 ± 0.17	
r_c	0.99 ± 0.02	
r_{pe}	0.93 ± 0.17	
r_e	0.82 ± 0.03	
r_{ph}	0.88 ± 0.02	

[1]LMMR, ln fecal egg count using the Modified McMaster method; LTCM, ln fecal egg count using the triple Chamber McMaster method.
[2]σ_a^2, σ_c^2, σ_{pe}^2, σ_e^2 are genetic, group of measurement, permanent environmental, and residual variances; h^2 and r^2 are the heritability and repeatability; r_g, r_c, r_{pe}, r_e, and r_{ph} are genetic, group of measurement, permanent environmental, residual, and total phenotypic correlations, respectively.

FAMACHA (Table 7) was positive and low with large standard error (0.24 ± 0.32). Higher genetic correlations were reported in literature. Álvarez et al (2018) reported genetic correlation between 0.55 and 0.78. Riley and van Wyk (2009) reported genetic correlation between FAMACHA and FEC, ranging from 0.73 to 0.85. van Wyk and Bath (2002) cited perfect correlation of 1.00. The different estimates for genetic correlations reported in the literature could be due to: 1) the level of infection, where higher worm environmental challenge leads to an increase in the heritability and genetic correlation estimates for FAMACHA and FEC traits; 2) data handling, specifically in relation with subjective clinical scoring for FAMACHA or the number of McMaster chambers counted; and 3) the different transformation methods of the raw FEC data in the different studies (e.g., Box–Cox transformation, log transformations, ln transformations; Riley and van Wyk, 2009; Silva et al., 2012; Balconi Marques et al., 2020). Based on the current results of estimated genetic correlations between LFEC and FAMACHA, there is no clear indication for usefulness for genetic selection for FAMACHA to genetically reduce the FEC in sheep. However, previous studies with Corriedale sheep found moderate genetic correlation estimates between Log_e (FEC + 100) and FAMACHA scores (0.55 ± 0.12) suggesting these two traits could be used together for selection toward more resilient or resistant sheep (Balconi Marques et al., 2020). In relation to resilience, it is possible for animals with low FAMACHA scores and high levels of FEC to be

Table 6. Estimates of variance components and genetic parameters for fecal egg count and other parasite resistance traits[1]

Estimate	LFEC	FAMACHA	BCS	WT
σ_a^2	0.31 ± 0.10	0.04 ± 0.03	0.07 ± 0.03	41.33 ± 8.15
σ_c^2	0.71 ± 0.21	0.09 ± 0.04	0.11 ± 0.05	103.14 ± 38.92
σ_{pe}^2	0.28 ± 0.09	0.08 ± 0.03	0.06 ± 0.02	16.03 ± 4.89
σ_e^2	1.20 ± 0.07	0.39 ± 0.02	0.17 ± 0.01	12.41 ± 0.78
h^2	0.12 ± 0.04	0.07 ± 0.05	0.17 ± 0.06	0.24 ± 0.07
r^2	0.23 ± 0.04	0.20 ± 0.04	0.30 ± 0.05	0.33 ± 0.08

[1]LFEC, integrated ln fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA, eye score system for red tint level of the color of the mucus membrane of the eyes, ranging from red "1" to "5" pale; BCS, body condition score; WT, body weight (kg); $\sigma_a^2, \sigma_c^2, \sigma_{pe}^2, \sigma_e^2$ are genetic, group of measurement, permanent environmental, residual variances; h^2 and r^2 are the heritability and repeatability.

Table 7. Estimates of genetic correlations ± SE (below diagonal) and phenotypic correlations (above diagonal) among parasite resistance traits[1]

	LFEC	FAMACHA	BCS	WT
LFEC	–	0.18	−0.13	−0.07
FAMACHA	0.24 ± 0.32	–	−0.25	−0.14
BCS	−0.03 ± 0.25	−0.02 ± 0.37	–	0.56
WT	0.22 ± 0.19	−0.01 ± 0.30	0.43 ± 0.17	–

[1]LFEC, integrated ln fecal egg counting records from LMMR and LTCM by prioritizing available LMMR records and fill missing records with re-scaled LTCM; FAMACHA, eye score system for red tint level of the color of the mucus membrane of the eyes, ranging from red "1" to "5" pale; BCS, body condition score; WT, body weight (kg).

tolerant to parasite infections and, therefore, FAMACHA could contain additional information beyond FEC. In addition, despite the low heritability of FAMACHA scores, collecting its records is less expensive and time consuming than individual FECs. Thus, large number of FAMACHA records could be generated rapidly and used for selection decisions. In addition, recording FAMACHA can be practical for managing anthelmintic resistance to drugs (in GIN populations) by reducing the number of treated animals in the flock (Ejlertsen et al., 2006; Burke et al., 2007; Reynecke et al., 2011; Maia et al., 2015). Therefore, FAMACHA is an important trait to record. Increasing the number of FAMACHA records would reduce the observed standard errors and increase the accuracy of estimation of genetic parameters, allowing to assess better its utility for breeding decisions. All other genetic correlations with LFEC were also low with large standard errors (Table 7). Therefore, based on these results, none of the traits in this study could be used as good genetic indicator for FEC. However, they may be used by producers as an indication of general health or parasite resilience of the sheep. The only moderate genetic correlation (r = 0.43 ± 0.17) was between BCS and WT (Table 7). Riley and van Wyk (2009) reported genetic and phenotypic correlations between BCS and WT of 0.47 and 0.59, respectively.

Higher estimates of correlation between traits were found due to permanent environmental (–0.57 to 0.54) and group of measurement (–0.67 to 0.89) effects (Supplementary Tables S1 and S2). For residual effect, the correlations were lower (–0.10 to 0.29; Supplementary Table S3), while the estimated phenotypic correlations varied between –0.13 and 0.56 (Table 7).

The genetic parameters estimated in this study were based on records from a single sheep flock, so they may not represent the diversity of flocks in Ontario and Canada. More representative estimates of genetic parameters will be calculated when additional data and potentially from different flocks become available.

Implications

The two fecal egg counting methods used, the Modified McMaster and the Triple Chamber McMaster, are highly correlated. However, they have different scales (means and variances), which should be taken into account when integrating their records. Heritability estimates for FEC and related indicator traits, such as FAMACHA, BCS, and WT were low to moderate, indicating that genetic progress for these traits is possible, but it will be achieved as a long-term goal. Genetic correlations between FEC and all indicator traits were low, indicating little to no benefit in using these traits as indicators for FEC. However, they may be used as indicators for general health and resilience to parasite load. The genetic parameter estimates may change when additional records from different sheep flocks become available.

Supplementary Data

Supplementary data are available at *Journal of Animal Science* online.

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Conflict of interest statement

The authors declare no real or perceived conflicts of interest.

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