



## Evaluation of the utility of subjective clinical parameters for estimating fecal egg counts and packed cell volume in Canadian sheep flocks

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### ABSTRACT

A study was conducted in sheep on Canadian farms to describe the relationship between packed cell volume (PCV) or fecal egg counts (FEC) and subjective clinical parameters that may indicate the severity of parasitic gastroenteritis. Twenty-one farms in Ontario (ON) and 8 farms in Quebec (QC) were purposively selected and visited during April–May (spring) and August (summer) 2007. At each farm visit, blood and fecal samples were collected from 10 ewes and 10 female lambs; body condition score (BCS), dag score (DS), fecal consistency score (FCS) and FAMACHA score were recorded for all sampled sheep. Packed cell volume was determined for all blood samples, and FEC were performed for all fecal samples. Summary statistics and simple correlations were performed for the parameters recorded. Two mixed models with random effects at the farm level were developed; one using PCV as the response variable and another using the natural log of eggs per gram of feces (lnEPG). Finally, the residuals from both models were correlated to the covariates in the models. The mean PCV values during the spring were 29.7% and 36.7% for lambs, and 28.8% and 31.1% for ewes, in ON and QC, respectively. During the summer, the mean PCV was 32.0% and 32.8% for lambs, and 30.1% and 29.9% for ewes, in ON and QC, respectively. The arithmetic mean FEC per gram of feces (EPG) during the spring was 3 and 2 for lambs, and 1266 and 789 for ewes, in ON and QC, respectively, whereas during summer the arithmetic mean EPG was 907 and 237 for lambs, and 458 and 246 for ewes, in ON and QC, respectively. Results from simple correlations indicated that PCV was negatively correlated with lnEPG ( $r = -0.255$ ;  $r^2 = 6.5\%$ ) and FAMACHA ( $r = -0.312$ ;  $r^2 = 9.7\%$ ), and positively correlated with BCS ( $r = 0.317$ ;  $r^2 = 10\%$ ). lnEPG was negatively correlated with BCS ( $r = -0.232$ ;  $r^2 = 5.4\%$ ) and PCV ( $r = -0.255$ ;  $r^2 = 6.5\%$ ), but positively correlated with FAMACHA ( $r = 0.178$ ;  $r^2 = 3.2\%$ ).

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and DS ( $r = 0.086$ ;  $r^2 = 0.7\%$ ). Results from the models indicated that PCV and InEPG residuals were negatively correlated with FAMACHA, FCS and almost all categories of BCS and DS, although the correlations were very low. The main results from this study suggested that none of the subjective clinical parameters evaluated were highly correlated with PCV or InEPG and therefore were not good predictors of InEPG or PCV on the studied farms in Ontario and Quebec.

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## 1. Introduction

Reliance on anthelmintic drugs as the only measure to control gastrointestinal nematodes (GIN) in sheep has led to the development of anthelmintic resistance (AR) (Prichard et al., 1980; Coles et al., 1994; Waller, 1997; Jackson and Coop, 2000; Falzon et al., 2013). Studies on the prevalence of AR, and risk factors associated with the development of AR, suggest that factors such as increased frequency of treatments and the use of clean pastures are likely the main contributors to the development of AR (Suter et al., 2004). Moving sheep to a “clean” pasture immediately after treatment of the flock with an anthelmintic drug increases the risk of selection for resistance as this markedly reduces the population of susceptible parasites in refugia. The term refugia refers to the parasite population that escapes exposure to the anthelmintic being applied, thus escaping selection for resistance (van Wyk, 2001; Silvestre et al., 2002), and refers primarily to the nematode population that is free-living on pasture as well as those residing in untreated animals (van Wyk, 2001). The risk of development of AR and its link to the need to preserve nematode populations in “refugia” underscores the necessity to validate targeted selective strategies to control GIN with reduced use of chemical drugs.

Exploiting the concept of “overdispersion”, where only a few animals carry the highest parasite burdens, has resulted in the development of several methods that target control toward this minority of highly infected sheep. Furthermore, decreasing the total number of annual treatments would theoretically help maintain a susceptible nematode population in refugia. Some studies have been carried out to identify clinical or production parameters which are strongly correlated with sheep GIN burden, and thus help focus treatments on heavily parasitized sheep with abnormal clinical parameters and/or lower production traits. To date, the main parameters studied as potential indicators of clinical GIN parasitism in sheep and goats are fecal soiling or dag score (DS) (Larsen et al., 1994; Osoro et al., 2007), fecal consistency score (FCS) (Larsen and Anderson, 2000), body condition score (BCS) (Osoro et al., 2007), and the more recently developed FAMACHA system that detects anemia caused primarily by *Haemonchus contortus* (Malan et al., 2001; van Wyk and Bath, 2002).

Studies conducted in Australia and the United Kingdom in lambs found poor correlations between DS and other markers for the presence of GIN such as fecal egg counts (FEC) or plasma pepsinogen concentrations (Larsen et al., 1994; Larsen and Anderson, 2000). However, a study conducted in 2003 in southwest England, in two to five month-old lambs exposed to natural parasite challenge,

demonstrated a significant positive association between DS and FEC, where lambs with higher DS had higher FEC (Broughan and Wall, 2007).

The use of the color of the palpebral conjunctivae of sheep for clinical evaluation of the level of anemia was explored by Malan et al. (2001) in South Africa. The aim was to identify individual sheep severely infected with *H. contortus* and selectively treat only those with clinical infections, i.e. anemic. This was to reduce the frequency of treatments, a risk factor for development of AR (Malan et al., 2001). In this study, ewes were primarily used to evaluate the FAMACHA concept: the authors reported that the Pearson correlation coefficients between hematocrit (packed cell volume, PCV) and mucous membrane anemia classifications varied between  $-0.3$  and  $-0.6$  ( $r^2 = 0.09$  and  $0.36$  respectively). Another study, conducted between 1998 and 2000 in South Africa, was carried out to validate the FAMACHA system in goats. This work, which used a hematocrit less than or equal to 19% to define anemia in goats, revealed that the sensitivity of the system to detect anemic goats improved from 31.1% when FAMACHA categories 4 and 5 were used to classify animals as anemic, to 80.0% when FAMACHA scores 3–5 were used, however, the specificity decreased from 91.2% to 54.3%, respectively (Vatta et al., 2001).

The FAMACHA system has been validated in sheep and goats in other regions of the world such as the south-eastern United States where anthelmintic resistance in *H. contortus* is a severe problem (Kaplan et al., 2004; Molento et al., 2009). The correlation between PCV and FAMACHA reported by Kaplan et al. (2004) for sheep was 0.52 ( $r^2 = 27.0\%$ ), but the correlation between FEC and FAMACHA was 0.21 ( $r^2 = 4.4\%$ ).

The objective of this study was to determine the relationship between PCV and eggs per gram of feces (EPG) with FAMACHA score, BCS, DS and FCS in sheep raised under cold continental climatic conditions in the provinces of Ontario (ON) and Quebec (QC) where parasite populations may be different from those described in warmer regions. The results will potentially allow for identification of clinical parameters that could be used as indirect markers of parasitic gastroenteritis in sheep.

## 2. Materials and methods

### 2.1. Study population and sampling protocols

This study was part of a 3-year longitudinal study (2006–2008) that has been described in detail elsewhere (Mederos et al., 2010). In short, the work reported here was conducted between April and August 2007 (second year

of the project) on 21 and 10 sheep farms in the Canadian provinces of Ontario (ON) and Quebec (QC), respectively.

The flocks were purposively selected, based on their willingness to participate in the study and to refrain from using anthelmintics except when treatment was necessary based on fecal egg counts. Among the 21 farms in ON, 6 were certified organic (CO), 9 were non-certified organic (NCO) and 6 were conventional (C). Among the 10 farms in QC, 2 were CO, 6 were NCO and 2 were C. In each flock, 10 ewes and 10 female grazing lambs were selected before pasture turnout. Female lambs were purposively selected because they would likely remain longer in the flocks than male lambs; a desirable feature for a 3-year longitudinal study. The ewes to be sampled were initially selected in 2006 following a systematic random process based on the order the sheep traveled through a handling system (e.g. every fifth ewe – depending on flock size). All measurements for this study were performed twice on the same animals: firstly at the beginning of the grazing season (April–May, 2007) and subsequently at the end of the summer (August, 2007). Hereafter, these will be referred to as “spring” and “summer” samplings. Multiple raters, who undertook training with the senior researcher SF, carried out the clinical scoring after having been determined to have an inter-observer agreement >80% by a Kappa test. Blood samples were obtained from each animal via jugular venipuncture into 10 ml vacutainer tubes containing 15% ethylenediamine tetra-acetic acid (EDTA) to determine the blood PCV. Fecal samples were collected directly from the rectum and examined individually to determine the FEC. Body condition scores were estimated and recorded for each sheep during the study period using a scale from 1 to 5 (Russel et al., 1969) (1 = emaciated; 5 = fat). Dag score, which is the accumulation of feces in the wool of the breech area, was assessed using a scale from 0 to 5 (0 = no dag; 5 = heavy dag) (Larsen et al., 1994), and a FCS was assessed with a scale from 1 to 5 (1 = hard pellets; 5 = watery) (Larsen and Anderson, 2000). A FAMACHA score, which measures the color of the eye mucosa as an indirect sign of anemia, was recorded using a FAMACHA chart (1 = red; 5 = white) (van Wyk and Bath, 2002).

## 2.2. Laboratory methods

Fecal samples were analyzed at the parasitology laboratory, Department of Pathobiology, University of Guelph. Fecal egg counts, expressed as eggs per gram of feces (EPG) were performed on individual fecal samples using a modified McMaster method described by Roepstorff and Nansen (1998), with a lower detection limit of 20 EPG. Coprocultures were carried out by weighing equal amounts of feces from each individual and pooling together by group at the farm level, i.e. group was defined as either ewes or lambs. This was done for each group and each farm for both spring and summer samples. The subsequent protocol followed was as described by Henriksen and Korsholm (1983) and modified by Fernández et al. (1999); cultures were maintained at room temperature for 14 days instead of using climatic chambers.

Packed cell volume values were determined using a Baxter Canlab microhematocrit centrifuge. Blood samples

were processed during a period of 24–48 h after collection, and fecal samples were processed during a period of 3–5 days after collection.

## 2.3. Statistical analysis

A dataset was built with EPG and PCV data, and the aforementioned clinical parameters that were collected from sheep in the spring and summer sampling. Summary statistics were generated using STATA/InterCooled 10.0 for windows (StataCorp., 2007). Spearman correlation coefficients were calculated for simple correlations between PCV or natural logarithm of EPG (lnEPG) against the categorical variables BCS, DS, FCS and FAMACHA, using SAS System for Windows version 9.1 (SAS Institute Inc., 2003).

Two mixed linear models were fitted ( $P < 0.05$ ), guided by a manual process using the PROC MIXED procedure in SAS. The main purpose of these models was to obtain the residuals to perform the correlations after removing the effect of the variables included as covariates (Muller and Fetterman, 2002). For the first model, PCV was the response variable. For the second model, lnEPG was the response variable; to normalize the distribution of the response variable and improve homoscedasticity. Univariate analyses were performed to establish the associations between PCV (first model) or lnEPG (second model) and BCS, DS, FCS, FAMACHA, season (spring and summer), age (ewes and lambs), province (ON and QC), and percentage of *Haemonchus* sp., *Teladorsagia* sp. and *Trichostrongylus* spp. infective larvae in feces (i.e. the main GIN genera obtained from coprocultures). All covariates with a  $P$ -value  $< 0.10$  were retained to build manual backward elimination multivariable models for PCV and for lnEPG. A random intercept parameter at the farm level was introduced to the models to adjust for the dependence of the data due to clustering of sheep within farms. Clustering of farms within province was controlled for by a fixed effect. The effect of potential confounders (e.g. sheep age, province and type of operation) was evaluated by removing them from the model and testing whether there was a  $\geq 30\%$  change in the coefficients of the main predictors (Dohoo et al., 2003). There was a diversity of breeds and cross-breeds in the study flocks (e.g. Suffolk, Dorset, Rideau, Romanov among others), therefore, breed variation was considered included within part of the farm variation and modeled as a random effect in the statistical model.

Two- and three-way interactions among significant predictors were tested by determining their significance at the 5% level ( $P < 0.05$ ).

From the two sets of residuals, partial correlation coefficients for PCV and for lnEPG were calculated. Residuals from both final models were also correlated against the model predictors using Spearman rank correlation statistics for categorical data.

Partial correlation coefficients ( $r_p^2$ ) were obtained to estimate the proportion of the residual variation explained by each of the covariates in the models, after taking into account the other variables in the models.

Since an exact statistical test could not be used to evaluate the presence of a common correlation between the

**Table 1**

Means values (and standard error in brackets) for packed cell volume (PCV), FAMACHA scores, eggs per gram of feces (EPG) and proportions of *Teladorsagia* sp., *Haemonchus* sp. and *Trichostrongylus* spp. in fecal samples, stratified by province, season and sheep age.

	PCV (%)	FAMACHA score	EPG	<i>Teladorsagia</i> sp. (%)	<i>Haemonchus</i> sp. (%)	<i>Trichostrongylus</i> spp. (%)
<b>Ontario (n = 21)</b>						
<i>Spring</i>						
Lambs	29.7 (0.4)	2.1 (0.1)	3 (0.5)	19.8 (1.4)	29.1 (2.3)	35.5 (2.2)
Ewes	28.8 (0.4)	2.4 (0.1)	1266 (203)	17.3 (1.2)	32.4 (2.1)	35.7 (2.1)
<i>Summer</i>						
Lambs	32.0 (0.4)	2.1 (0.1)	907 (218)	25.9 (2.3)	31.2 (3.0)	30.1 (2.1)
Ewes	30.1 (0.4)	2.4 (0.1)	458 (83)	18.7 (1.9)	31.1 (2.6)	30.9 (2.0)
<b>Quebec (n = 10)</b>						
<i>Spring</i>						
Lambs	36.7 (0.4)	1.0 (0.02)	2 (0.8)	30.9 (3.9)	36.6 (5.0)	9.7 (0.9)
Ewes	31.1 (0.5)	1.4 (0.1)	789 (155)	31.0 (3.2)	35.3 (3.5)	16.8 (2.3)
<i>Summer</i>						
Lambs	32.8 (0.5)	1.1 (0.03)	237 (124)	13.5 (2.7)	48.0 (6.8)	10.5 (1.3)
Ewes	29.9 (0.4)	1.5 (0.8)	246 (76)	16.4 (2.6)	42.8 (4.0)	23.4 (2.5)

categorical variables and residuals from both models, an approximate analysis of the correlation homogeneity was performed using Fisher's z-transform and by adjusting the *P*-values for multiple comparison groups (Sokal and Rohlf, 1995).

### 3. Results

#### 3.1. Descriptive statistics

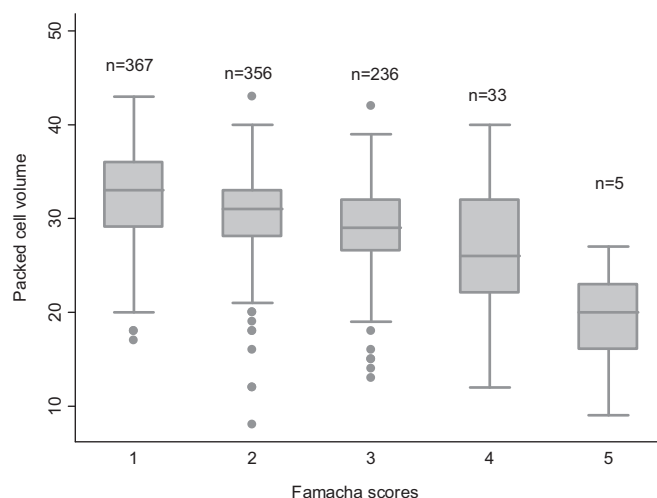
Summary statistics for PCV, FAMACHA, EPG, percentage of *Teladorsagia* sp., *Haemonchus* sp. and *Trichostrongylus* spp. are presented in Table 1, averaged over province, season and sheep age.

Mean PCV values were >19%, the cutpoint considered to determine whether anemic or normal (Vatta et al., 2001; Burke et al., 2007). The proportion of PCV values ≤19% in both samplings was 1.9% and 3.2% for ewes and lambs respectively. Nevertheless, lambs had significantly

higher PCV values than ewes ( $P < 0.05$ ) except for the spring sampling in ON where the difference was not significant ( $P > 0.05$ ). The mean FAMACHA score results were in the range of red (FAMACHA = 1) to red-pink (FAMACHA = 2) for sheep in the two provinces in both seasons, indicating that anemia was generally not a major problem in the study population. The ewe group generally had higher mean EPGs in the spring, while lambs generally had higher mean EPGs during the summer.

Results from fecal cultures showed that the percentage of *Haemonchus* sp. was higher for the QC lambs and ewes during summer 2007 compared with the same groups in ON ( $P = 0.012$  and  $P = 0.014$ , respectively), as presented in Table 1. The proportion of farms with *Haemonchus* sp. was 84% and 73% in spring and summer respectively.

Fig. 1 shows the distribution of PCV values for each FAMACHA score for all sheep in the study, for both seasons and all farms in ON and QC combined.



**Fig. 1.** Box and whisker plots showing the relationship between packed cell volume and FAMACHA scores for all sheep sampled in spring and summer from Ontario and Quebec farms. The central line in the box represents the median, and the upper and lower lines of the box represent 25th and 75th percentiles, respectively, while the whiskers above and below the box represent an "inner fence", reflective of the 99th and 1st percentiles, respectively. The circles represent outlier observations.

**Table 2**Arithmetic mean fecal egg counts in eggs per gram of feces (EPG) for each FAMACHA score category, by sheep age and season, for all farms ( $n = 31$ ).

FAMACHA	Spring		Summer	
	Ewes mean EPG (range) <i>n</i>	Lambs mean EPG (range) <i>n</i>	Ewes mean EPG (range) <i>n</i>	Lambs mean EPG (range) <i>n</i>
1	639 (0–9140) <i>n</i> = 92	2 (0–40) <i>n</i> = 91	158 (0–1220) <i>n</i> = 79	427 (0–11160) <i>n</i> = 85
2	722 (0–8580) <i>n</i> = 119	3 (0–40) <i>n</i> = 63	282 (0–2440) <i>n</i> = 103	571 (0–6800) <i>n</i> = 66
3	1443 (0–14920) <i>n</i> = 60	3 (0–20) <i>n</i> = 46	735 (0–10540) <i>n</i> = 87	829 (0–8900) <i>n</i> = 40
4	3027 (0–12740) <i>n</i> = 11	7 (0–20) <i>n</i> = 3	360 (0–1880) <i>n</i> = 12	4462 (0–26180) <i>n</i> = 8
5	12,920 (7200–21160) <i>n</i> = 4	<sup>a</sup>	<sup>b</sup> <i>n</i> = 1	<sup>a</sup>

<sup>a</sup> No observations in this category.<sup>b</sup> Only 1 observation, therefore, there is no mean.**Table 3**Spearman's rank correlation coefficients and correlation coefficients squared ( $r^2$ ), expressed as percentages, and  $P$ -values, for the association between packed cell volume (PCV) and natural logarithm of eggs per gram of feces (lnEPG) and other covariates from the univariate analyses.

	PCV		lnEPG	
	$r$ ( $r^2$ )	$P$ -value	$r$ ( $r^2$ )	$P$ -value
lnEPG	−0.255 (6.5)	<0.001	1.000 (1.0)	–
FAMACHA <sup>®</sup> score	−0.312 (9.7)	<0.001	0.178 (3.2)	<0.001
BCS <sup>a</sup>	0.317 (10.0)	<0.001	−0.232 (5.4)	<0.001
DS <sup>b</sup>	0.004 (0.0)	0.888	0.086 (0.7)	0.007
FCS <sup>c</sup>	−0.029 (0.0)	0.369	0.012 (0.0)	0.695
PCV	1.000 (1.0)	–	−0.255 (6.5)	<0.001

<sup>a</sup> Body condition score.<sup>b</sup> Dag score.<sup>c</sup> Fecal consistency score.

The mean BCS's were slightly lower for the ewe groups than for the lamb groups in both provinces and in both seasons. However, the mean differences between seasons were significant only for the ewe groups in both provinces ( $P < 0.05$ ) (Table II, Annex I).

Mean EPG results grouped by FAMACHA scores, age and season suggested a tendency for higher EPG counts with FAMACHA = 3–5 compared to FAMACHA = 1–2 (see Table 2). Univariate Spearman's rank correlation coefficients between PCV and FAMACHA scores were negative ( $P < 0.001$ ). In contrast, a positive correlation, although quite small, was observed between PCV and BCS ( $P < 0.001$ ), as indicated in Table 3. The univariate correlation coefficients between lnEPG and FAMACHA score or DS were both positive ( $P < 0.05$ ). In contrast, a negative correlation was observed between lnEPG and BCS ( $P < 0.05$ ), however, the correlation was low.

### 3.2. Statistical models

The estimates for the variance components at the different levels of the hierarchical structure obtained from the two random effect models with PCV and lnEPG as response variables are presented in Table 4. Most of the variation in PCV and lnEPG occurred within sheep (i.e. there was very

little clustering of farms within provinces or sheep within farms).

Partial correlation coefficient percentages of the fixed effects from the final two models with PCV and lnEPG as response variables are presented in Table 5.

Results from the final model with PCV as the response variable indicated that the covariates age, province, FAMACHA, BCS, FCS, DS, season, operation type, and the interactions between age x FAMACHA, season x province

**Table 4**

Summary of the variance components and proportions explained at the different levels of the hierarchy, obtained from models with packed cell volume (PCV) and natural logarithm of eggs per gram of feces (lnEPG) as dependent variables – all sheep and both seasons.

Covariance parameters	Variance estimate	Lower 95%CI	Upper 95%CI	Variance explained <sup>a</sup> (%)
<i>PCV model</i>				
Farm level	1.52	0.78	4.09	7.7
Sheep level	3.24	1.96	6.31	16.5
Residual	14.93	13.09	17.10	75.8
<i>lnEPG model</i>				
Farm level	1.07	0.60	2.37	14.5
Sheep level	0	0	0	0
Residual	6.30	5.75	6.92	85.5

<sup>a</sup> Percent of total variance explained at the various levels.

**Table 5**

Partial correlation coefficients squared and expressed as percentage ( $r_p^2$ ) showing the percentage that each covariate contributed to the residual variation for the packed cell volume (PCV) and natural log of eggs per gram (lnEPG) models.

Effect	PCV		lnEPG	
	$r_p^2$ (%)	P-value	$r_p^2$ (%)	P-value
Age <sup>a</sup>	2.20	0.004	8.87	<0.001
Province <sup>b</sup>	15.9	0.052	7.37	0.277
FAMACHA	3.70	<0.001	1.51	0.046
BCS <sup>c</sup>	12.73	<0.001	6.44	<0.001
FCS <sup>d</sup>	2.21	0.004	0.24	0.831
DS <sup>e</sup>	1.70	0.013	3.34	0.001
Season <sup>f</sup>	0.57	0.246	2.24	0.003
Operation type <sup>g</sup>	0.65	0.493	0.79	0.126
Age * FAMACHA	2.34	<0.001	0.85	0.588
Season * province	11.5	<0.001	3.39	0.001
Season * operation	1.79	0.004	1.69	0.012

FAMACHA = short for FAMACHA score.

<sup>a</sup> Ewes versus lambs.

<sup>b</sup> Ontario versus Quebec.

<sup>c</sup> Body condition score.

<sup>d</sup> Fecal consistency score.

<sup>e</sup> Dag score.

<sup>f</sup> Summer versus spring.

<sup>g</sup> Certified organic, non-certified organic and conventional.

and season x operation type contributed approximately 50% of the unexplained variation in PCV (Table 5). For the second model with lnEPG as the response variable, the partial correlation coefficients indicated that age, province and BCS were the covariates that most contributed to the proportion of unexplained variance in lnEPG, as shown in Table 5.

The infective stages of the predominant nematodes *Teladorsagia* sp., *Haemonchus* sp. and *Trichostrongylus* spp., expressed as a percentage, were not significant predictors of PCV or lnEPG ( $P > 0.05$ ) and therefore were removed from the final models.

The relationship between the PCV and lnEPG residuals, obtained from the random effect models, is presented in Figure 1, Annex I. The Spearman's correlation coefficients indicated that the intrinsic correlation between PCV and lnEPG residuals was negative and significant, even though the intensity of the relationship was small ( $r^2 = 1.9\%$ ).

The Spearman's correlation coefficients between the residuals from the models, by each category of the different covariates, are presented in Table III Annex I. Approximate analysis for homogeneity of the correlations between the different variable categories indicated that categories of the covariates 'age' (ewes versus lambs), 'province' (ON versus QC), 'FAMACHA' (scores 2 versus 4, and 3 versus 4) and 'BCS' (scores 2 versus 4) did not share a common correlation ( $P < 0.01$  after adjusting for multiple comparisons).

#### 4. Discussion and conclusions

The results presented above demonstrated that none of the proposed indicators for identifying sheep carrying heavy gastrointestinal nematode burdens have a strong linear relationship with either lnFEC or PCV in this study.

Although low, the positive correlation coefficients obtained by univariate Spearman correlations for lnEPG and FAMACHA, as well as the positive correlation for BCS

and PCV, are biologically sensible (Table 3) since higher PCV values are expected when EPGs and FAMACHA scores are low, and vice versa. The correlations obtained between lnEPG and both DS and FCS are in concordance with results presented by Pollot et al. (2004), who studied genetic and phenotypic correlations in Merino sheep in Australia and found low correlations between FEC and DS or FCS (0.02 and  $-0.03$  for DS and FCS, respectively).

The univariate correlation coefficient obtained between lnEPG and FAMACHA in our study ( $r = 0.18$ ) was not different from the one presented by Kaplan et al. (2004) who reported a correlation of 0.21 for sheep in the southern United States, meaning that FAMACHA explained approximately 4.4% of the variation in FECs ( $r^2 = 0.21^2 = 0.044$ ). However, the univariate correlation between PCV and FAMACHA from our study ( $r = -0.312$  and  $r^2 = 0.097$ ) was substantially lower than the one reported by those authors (Kaplan et al., 2004), who found a correlation of  $-0.52$  for sheep ( $r^2 = 0.270$  or 27%).

Simple associations do not reflect the relationship between two or more variables in a population (Sokal and Rohlf, 1995); when we removed the possible confounding effects of other covariates in our correlation model, partial correlation coefficients indicated that FAMACHA explained an even lower percentage of the variation of PCV and lnEPG (note the  $r_p^2$  for FAMACHA and the interaction variable Age \* FAMACHA in Table 5). The Kaplan et al. (2004) study carried out univariate correlations without controlling for confounding for comparison purposes.

In the work reported here, Spearman's correlation coefficients between the residuals from both models (PCV and lnEPG) indicated that there was a significant correlation between the residuals for the different levels of BCS, DS, FCS and FAMACHA. However, the intensity of the correlation was very low, except for FAMACHA score 4 ( $r^2 = 40.5\%$ ). This is in concordance with results presented from the summary statistics (Table 1), that indicated there were few truly anemic sheep in the study population, since the mean PCV values fell in the normal range ( $\geq 19\%$ ) for both sheep ages during the spring and summer samplings. Furthermore, the mean FAMACHA scores were generally in the normal range of red and red-pink colors for the eye mucosa (1.1–2.4). However, summary FECs suggested that the ewe groups tended to have higher mean FECs and lower PCV values during the spring sampling compared with the summer, which could be related to parturition and lactation of the ewes during that period of time, and the associated down-regulation of immunity and increased susceptibility to parasites (Gibbs, 1986; Beasley et al., 2010; Knight et al., 2010). The distribution of PCV values by FAMACHA scores suggested a decrease in PCV as FAMACHA score increased (Table 3 and Fig. 1), as expected. However, there was a wide dispersion in the distribution of PCV values at each of the FAMACHA scores in our study, suggesting that the FAMACHA parameter is less reliable at the animal level than at the population level, which may be a function of the small number of sheep with low PCVs in this study.

Similar studies to contrast the findings in the present study were not found in the literature.

In conclusion, FAMACHA score, BCS, and DS were significantly correlated with PCV or lnEPG, and FCS was

significantly correlated with PCV (Table 5). However, none of the clinical parameter associations that were tested were sufficiently strong enough to accurately estimate PCV or FECs under the conditions present on the farms in ON or QC that participated in this study. As such, they cannot be used to reliably quantify the level of GIN egg output in sheep in the studied regions. Body condition scores had the highest partial correlation coefficients of all the clinical parameters, accounting for 12.7% of the residual variation in the PCV model. Lastly, it should be recognized that areas with a cold continental climate in which *Haemonchus* sp make up only a moderate proportion of the GIN species identified, appear to have different GIN epidemiology than tropical and subtropical areas. In contrast, in warm climates which are more suitable for the development and survival of *H. contortus*, or in specific situations when *Haemonchus* is the predominant parasite due to widespread anthelmintic resistance, the FAMACHA system could be a suitable tool.

### Conflicts of interest

The authors declare no conflict of interests and disclose no financial relationship with people or organizations that could bias this work

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### Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vetpar.2014.08.030>.

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