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## Early detection of *Haemonchus contortus* infection in sheep using three different faecal occult blood tests

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### Abstract

*Haemonchus contortus* is a blood-sucking parasite causing the presence of faecal occult blood (FOB). The objective was to study three different FOB tests in order to have a new indicator of *H. contortus* infection in sheep that could be included in the genetic evaluation system as an alternative selection criterion to faecal worm egg count (FEC). A total of 29 Corriedale lambs were experimentally infected with 10.000 larvae of *H. contortus*. Stool samples were recorded for FEC and FOB tests (Hexagon, Hematest<sup>®</sup> and Multistix<sup>®</sup>), blood for packed cell volume (PCV), haemoglobin, white and red blood cell count (RBC), and FAMACHA<sup>®</sup> for scoring anaemia. At the end of the experiment lambs were slaughtered to worm burden count. Field infection was achieved in 309 Merino lambs under natural parasite challenge. FEC data were normalized through logarithmic transformation (LnFEC). Pearson correlation was estimated to examine the relationship between all traits. The three tests were able to detect the presence of FOB at day 11. FEC, PCV and RBC decreased to sub-normal values from day 18. FAMACHA<sup>®</sup> score 3 was considered to be indicative of anaemia. Most of the correlations were of high magnitude, with the exception of Multistix<sup>®</sup> test that was moderately correlated with haematological parameters, LnFEC and FEC. In field infection, most samples were negative to FOB tests and the correlations were lower than those calculated under experimental infection. In conclusion, FOB tests were able to detect haemonchosis earlier than FEC under high experimental parasite challenge. However, they were not able to detect FOB under natural mixed parasite challenge. FAMACHA<sup>®</sup> and PCV demonstrated to be good indicators of Haemonchosis, having moderate to high correlations with FEC.

**Keywords:** Faecal occult blood test, Faecal worm egg count, Haemonchosis, Sheep.

### Introduction

Gastrointestinal (GI) parasitism is one of the most important diseases in sheep production (Perry *et al.*, 2002), causing important losses by a decrease in the production and by the costs of control measures. Faecal worm egg count (FEC) using the modified McMaster technique, is currently the main method used in laboratories for the diagnosis of GI parasite infection and to determine the need for anthelmintic treatment. Furthermore, FEC has been considered as an indicator of resistance to nematodes and is the most commonly used selection criterion when selecting animals for enhanced GI parasite resistance (Bishop, 2011). However FEC has a disadvantage since it is unable to indicate a worm burden until egg output commences at three to four weeks after infection, a time at which the worms are well established and the host may already be suffering adverse effects (Kahn and Watson, 2001).

*Haemonchus contortus* (Barbers Pole worm) is the most predominant parasite genus present in Uruguay (Nari *et al.*, 1977; Castells, 2009). It is a blood-feeding parasite of the abomasum of sheep, and may cause high morbidity and mortality rates in a flock. The average

blood loss per worm per day is around 0.05 ml (Clark *et al.*, 1962), thus if an animal is infested by 5000 *Haemonchus* spp., it may have a daily loss of 250 ml of blood, that will produce a severe anaemia in a short period of time.

In the 90's, a novel system called FAMACHA<sup>®</sup> was developed in South Africa, which enables clinical identification of anaemic sheep and goats (Bath *et al.*, 1996). Since anaemia is the primary pathologic effect from infection with *H. contortus*, this system can be an effective tool for identifying those animals that require treatment (but only for this parasite) (Kaplan *et al.*, 2004). Moreover, *H. contortus* starts feeding at day 11 post-infection, causing the presence of blood in the host's faeces (Colditz and Le Jambre, 2008). Since in *Haemonchus* spp. infections there is a strong correlation between blood loss and both worm burdens and worm egg production, it appeared that methods to detect haemoglobin or other blood products in sheep faeces would be a useful means of assessing infection levels (Le Jambre, 1995). Although faecal occult blood (FOB) test was developed as an initial screening test for the detection of colorectal cancer in human medicine (Wu *et al.*, 2009; Jenkinson and Steele, 2010), there are

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various causes of positive FOB including infection with some GI parasites. As the presence of blood in faeces precedes the commencement of egg production (day 18 post-infection), FOB test might provide a leading or predictive indicator of the severity of GI parasite infection in comparison to the usual information provided by FEC (Colditz and Le Jambre, 2008). The aim of the present work was to study three different FOB tests in order to have a new indicator of haemonchosis in sheep that gives the possibility to be included in the genetic evaluation system as an alternative selection criterion to FEC.

### Material and Methods

#### *Animals and data collection*

##### *Experimental challenge*

The first part of the study was carried out at “La Magnolia”, an experimental station of the National Institute of Agricultural Research (INIA) located in the northern part of Uruguay, between May and June (end of autumn) of 2010. Records were obtained from 29 six-month-old castrated male lambs, which were bought from a Corriedale commercial flock. Before the beginning of the experiment, animals were drenched orally with 2.5 mg/kg ZOLVIX® (Monepantel) in order to eliminate natural infections that might be present. Ten days later they were checked to verify that the treatment had been effective. Since some parasites as liver Fluke and Coccidia can lead to positive FOB test results, Willis (1921) and Happich and Boray (1969) techniques were assessed to discard the presence of these agents, respectively. It is well known that adult stages of liver fluke have blood-sucking activities (Soulsby, 1987) and can give rise to the appearance of blood in faeces. Similarly, coccidiosis produce diarrhoea streaked with blood in young sheep (Soulsby, 1987). Additionally, complete haemograms of each animal were made one week before beginning the experiment, in order to discard concurrent infections that cause faecal blood loss (*e.g.* bacterial enteritis) that could interfere with the results of the present study. Animals were kept under housing system, not having access to natural pastures to avoid interferences with other parasites. They were fed with a diet based on 70% corn and 30% sorghum, bales of alfalfa and water *ad libitum*. There was an adaptation period to the diet before the beginning of the experiment. During this period, animals were tested in order to discard false positive results by the interference of plant peroxidases with guaiac-based FOB tests (Sinatra *et al.*, 1999).

Lambs were experimentally infected with 10.000 larvae (L3) of *H. contortus per os*. Total L3 were administered in two doses, 5000 larvae on day 1 of the experiment and 5000 larvae 48 hours later.

Animals were individually sampled for the following determinations: (a) Stool samples for FEC and FOB tests: FEC were determined using a modified McMaster

technique (Whitlock, 1948), where each egg observed represented 100 eggs per gram of faeces. The three FOB tests used for the detection of blood in faeces were Hexagon OScreen®, Hematest® and Multistix®. (b) 2.5 ml of blood collected by jugular venepuncture in EDTA vacutainers, to measure packed cell volume (PCV), haemoglobin (Hb), white blood cell count (WBC) and red blood cell count (RBC). WBC and RBC were determined using a Neubauer chamber cell counting. (c) FAMACHA® score on a 5-point scale for scoring anaemia on the basis of the colour of the conjunctiva membranes.

FEC, FOB tests, FAMACHA® and PCV were evaluated twice a week while Hb, WBC and RBC once a week, for a month.

At the end of the experiment (day 40), lambs were slaughtered and their worm burdens were recorded. However, if before day 40 animal welfare could be compromised (*e.g.* high FEC, FAMACHA® score 5, severe clinical signs), it was proceeded immediately to slaughter the animal. Final FEC samples were collected prior to euthanasia. Animals were kept in “La Magnolia” during all the experiment, being slaughtered at the same place, not being involved any travelling system.

This experiment was approved by the Honorary Committee of Animal Experimentation (CHEA) of the University of the Republic of Uruguay.

##### *Field infection*

In 2011, field infection was achieved in the Fine Merino Nucleus, a flock belonging to “Glencoe”, another research station of INIA (Latitude 32°00' S and longitude 57°08' W). Records were obtained from 309 lambs, 177 males and 132 females, under natural mixed-species parasite challenge on pasture. On May 12, individuals were sampled for FEC (n=291), PCV (n=306), FAMACHA® (n=309) and FOB tests (n=290), having on average 7.6 months-old, corresponding this date with the first FEC sampling, according to the protocol to evaluate resistance to GI parasite in Uruguay (Goldberg *et al.*, 2011). It consists of sampling 10 to 15 naturally-infected post-weaning lambs randomly selected every two weeks, until FEC mean is 500 and no more than 10% of individuals have FEC values of zero. At that moment, all animals are sampled obtaining the first record for FEC (FEC1). They are dewormed immediately and the same process is repeated until the second record for FEC (FEC2) is obtained. Only records corresponding to FEC1 were taken into account for the analysis in the present study. Faecal cultures of infective larvae were also prepared in order to assess the species composition of nematode infection at that time.

##### *Faecal occult blood tests*

Each stool sample was processed to detect occult blood using three different tests. All of them are based on the peroxidase-like activity of haemoglobin in catalyzing the oxidation by peroxide of a chromogen.

### **Hexagon OBScreen (Human, Germany)**

This test consists in a guaiac impregnated paper enclosed in a cardboard frame. A positive reaction is indicated by the appearance of a blue colour 30 seconds after addition of the developer reagent to the faecal sample. This test detects 2 ml of blood per 100g stool.

### **Hematest® (Siemens, USA)**

The specimen is smeared onto a filter paper supplied with Hematest® Reagent Tablets. A tablet is placed on the filter paper and moistened with water. The presence of haemoglobin in the specimen is indicated by development of a blue colour on the filter paper surrounding the tablet. This test detects 4 ml of blood per 100g stool.

### **Multistix® (Siemens, USA)**

It is a firm plastic strip joined with a reagent area that tests for occult blood in urine. An aliquot of the faecal sample was diluted (1/500) and five millilitres of this final solution was boiled for 20 minutes before testing. The resulting colour ranges from orange through green. This test provides a quantitative scale between 1 (absence of blood) and 4 and has a sensitivity of 0.15 to 0.62 µg/ml haemoglobin.

### **Statistical analysis**

For FEC, a transformation to  $\log_e(\text{FEC}+100)$  (LnFEC) was used to normalize distribution of data before analysis. LnFEC, FEC, PCV, Hb and RBC were analysed by ANOVA for the effect of FAMACHA® and Multistix® score, using the General Linear Models (GLM) Procedure of SAS (Statistical Analysis System, Version 9.2, 2008). Pearson correlation coefficients were estimated through CORR procedure of SAS (Statistical Analysis System, Version 9.2, 2008), to examine the relationship between LnFEC, FEC, PCV, Hb, RBC, FAMACHA® and Multistix®. The correlation coefficient was also calculated between LnFEC and FEC with total worm burden.

## **Results**

### **Artificial challenge**

In Table 1 are represented the changes of blood parameters, FEC, FAMACHA® and Multistix® test over time. Total WBC was constant amongst the measurement period and within the reference values (5000-6000 leucocytes/mm<sup>3</sup>), demonstrating the absence of concomitant infections that could interfere with the experiment. Values of PCV, RBC and Hb decreased to subnormal values since day 18 post-infection, showing the haematological effects of *H. contortus* infection.

The three tests were able to detect the presence of occult blood in the stool at 11 days post-infection. The mean values in days of each FOB test to become positive were: 12.2, 12.7 and 12.0 for Hexagon OBScreen®, Hematest® and Multistix®, respectively; having the three tests a minimum value at 11 and a maximum at 18 days. The mean value in days for FEC was 18.9,

having a minimum value at 14 and a maximum at 25 days. At day 11, Hematest® detected occult blood in 59% of the animals, Hexagon in 69% and Multistix® in 76% of them; while FEC of all the animals was lower than 100. At day 14, 93% were positive for Hexagon and Multistix® and 83% were positive to Hematest® and FEC mean continued to be lower than 100. At day 18, 100% were positive for Hexagon and Multistix® and 97% were positive to Hematest® and FEC increased with a mean of 417 eggs per gram (epg). In the present study, Hematest® was the test with the lowest sensitivity, since two animals that were positive on day 11 were then negative on day 14, and one lamb which was positive on day 14 became negative on day 18. This result was not observed with the other tests, although there were changes in Multistix® scores in some animals. Three lambs had score 3 on day 11 and score 2 on day 14. Additionally, four animals with score 3 on day 14, became 2 on day 18.

FAMACHA® score results in each measurement time point showed an increase of the higher scores over time. On day 5, animals had FAMACHA® scores of 1 or 2, while on day 25, 26 lambs (90%) had score  $\geq 3$ . The mean value on day 18 was 2.9 (Table 1). In Table 2 are presented the means of Multistix® test for each FAMACHA® score.

As shown in Table 3, FAMACHA® scores 1 or 2 were not statistically different ( $p > 0.05$ ) for FEC, PCV, Hb and RBC. A FAMACHA® score of 3 was considered to be indicative of anaemia, corresponding to a mean FEC of 1758 epg, a mean PCV value of 23% and a mean Hb value of 6.3 g/dl. For Multistix® test, only score 1 was considered statistically different from scores 2, 3 and 4 for LnFEC, and from scores 2 and 3 for FEC, PCV, Hb and RBC (Table 4). Thus, although it is a quantitative test, it performed as qualitative. Scores 1 and 4 did not show statistically significant differences ( $p > 0.05$ ) for FEC, PCV, Hb and RBC because of the small amount of records with Multistix® score 4 and the large standard error. However, great differences can be observed between Least Square Means values for FEC and haematological parameters for score 1 and for score 4. All the correlations estimated were significantly different from zero ( $p < 0.001$ ) (Table 5).

Most of the correlations were of high magnitude, with the exception of Multistix® test that was moderately correlated with haematological parameters, LnFEC and FEC. The high correlations between FAMACHA® score with all traits show that FAMACHA® system is a good indicator of *Haemonchus* spp. infection.

Some animals had to be slaughtered before the end of the experiment (10 lambs on day 28 and seven on day 34) for the reasons explained in Material and Methods section. The correlation between worm burden and FEC was 0.67 (0.39-0.83) (95% confidence interval) and 0.68 (0.41-0.84) with LnFEC.

**Table 1.** Descriptive statistics for the changes in packed cell volume (PCV, %), haemoglobin (Hb, g/dl), red blood cell count (RBC, erythrocytes/mm<sup>3</sup>), white cell count (WBC, leucocytes/mm<sup>3</sup>), faecal worm egg count (FEC, epg), FAMACHA<sup>®</sup> and Multistix<sup>®</sup> after experimental *H. contortus* infection (10.000 L3).

Day PI	PCV	Hb	RBC	WBC	FEC	FAMACHA	Multistix
0							
n	29	29	29	29	29	29	29
Mean	28.3	7.8	8.8×10 <sup>6</sup>	5919	0	1.7	1
sd	5.3	1.6	1.6	2421	0	0.5	0
5							
n	-	-	-	-	29	29	29
Mean	-	-	-	-	0	1.2	1
sd	-	-	-	-	0	0.4	0
8							
n	28	0	0	0	29	29	29
Mean	30.4	-	-	-	0	1.8	1
sd	3.8	-	-	-	0	0.8	0
11							
n	29	29	29	29	29	29	29
Mean	34.2	8.9	9.7×10 <sup>6</sup>	5907	0	2	1.9
sd	5.1	1.3	1.8	2153	0	0.7	0.7
14							
n	27	0	0	0	29	29	29
Mean	26.5	-	-	-	3.4	2.7	2.5
sd	5.6	-	-	-	18.6	0.6	0.6
18							
n	29	29	29	29	29	29	29
Mean	24.8	6.8	7.2×10 <sup>6</sup>	6128	417.2	2.9	2.8
sd	6.9	1.7	1.9	1857	618.8	0.8	0.7
20							
n	29	0	0	0	29	29	29
Mean	16.5	-	-	-	748.3	3.1	2.6
sd	5.4	-	-	-	921.4	1.1	0.6
25							
n	29	29	29	29	29	29	29
Mean	15.7	4.5	5.0×10 <sup>6</sup>	5107	5266	3.5	2.4
sd	5.5	1.6	1.5	2004	4387	0.9	0.5

Day PI: Day post-infection, n: Number of observations, sd: Standard deviation.

### Field infection

Faecal culture results (from FEC1 sampling) showed that *Haemonchus* spp. was the most prevalent parasite genus (68%) followed by *Trichostrongylus* spp. (20%), with other genera less abundant. The 89% of records were negative with Multistix<sup>®</sup> (n=258) and only a few were positive with a score of 2 (n=32). All the samples that were negative with Multistix<sup>®</sup> were also negative with Hexagon test. Among the 32 samples with score 2, 17 were positive and 15 negative with Hexagon test.

Since Hematest<sup>®</sup> was the test with the lowest sensitivity in the first part of the experiment and most samples were negative for the other two tests, it was decided not to perform this test. The score 1 of Multistix<sup>®</sup> test was statistically different (p<0.05) from score 2 for LnFEC and FEC, but not for PCV.

From 306 PCV records, only 21 had sub-normal values (i.e. PCV<27%). From 309 FAMACHA<sup>®</sup> records, 122 had score 1, 141 score 2 and 46 score 3. Therefore, under natural infection, scores 4 and 5 were not

observed. For the traits FEC and LnFEC, there were no statistically significant differences ( $p > 0.05$ ) between scores 1, 2 and 3 of FAMACHA<sup>®</sup>. However, for PCV, Least Square Means showed statistically significant differences ( $p < 0.01$ ) between the three FAMACHA<sup>®</sup> scores analyzed.

As is shown in Table 6, the correlation between FEC and LnFEC was higher than under experimental

**Table 2.** Means of Multistix<sup>®</sup> test for each FAMACHA<sup>®</sup> score and the number of records observed for each score.

FAMACHA <sup>®</sup>	Multistix <sup>®</sup>					Mean (s.d)
	1	2	3	4	Total	
1	48	3	1	0	52	1.09 (0.36)
2	40	23	23	3	89	1.88 (0.92)
3	8	29	20	1	58	2.24 (0.71)
4	0	11	12	1	24	2.58 (0.58)
5	0	2	7	0	9	2.78 (0.44)
Total	96	68	63	5	232	

**Table 3.** Least square means (s.e: standard error) from ANOVA analysis and number of records in brackets [ ] of faecal worm egg count (FEC), FEC logarithmically transformed (LnFEC), packed cell volume (PCV), haemoglobin (Hb) and red blood cell count (RBC) for the five scores of FAMACHA<sup>®</sup> system under experimental *H. contortus* infection (10.000 L3).

FAMACHA <sup>®</sup>	LnFEC (Ln epg)	FEC (epg)	PCV (%)	Hb (g/dl)	RBC (cells $\times 10^5$ )	Worm burden
1	4.61 <sup>a</sup> (0.17) [52]	0 <sup>a</sup> (300) [52]	31.17 <sup>a</sup> (1.08) [30]	8.68 <sup>a</sup> (0.37) [17]	9.17 <sup>a</sup> (0.41) [17]	-
2	5.11 <sup>b</sup> (0.13) [94]	316 <sup>a</sup> (223) [94]	28.99 <sup>a</sup> (0.66) [81]	8.14 <sup>a</sup> (0.22) [48]	9.07 <sup>a</sup> (0.25) [48]	1980; 5960 [4]
3	6.41 <sup>c</sup> (0.14) [81]	1758 <sup>b</sup> (241) [81]	23.24 <sup>b</sup> (0.79) [56]	6.33 <sup>b</sup> (0.27) [31]	6.89 <sup>b</sup> (0.31) [31]	4800; 8710 [12]
4	7.32 <sup>d</sup> (0.22) [30]	3057 <sup>c</sup> (395) [30]	15.02 <sup>c</sup> (1.21) [24]	4.43 <sup>c</sup> (0.39) [15]	4.86 <sup>c</sup> (0.44) [15]	5160; 8950 [6]
5	8.06 <sup>d</sup> (0.35) [12]	5083 <sup>d</sup> (625) [12]	9.40 <sup>d</sup> (1.98) [9]	2.50 <sup>d</sup> (0.68) [5]	3.00 <sup>d</sup> (0.76) [5]	5510; 8390 [7]

\*Different superscript letters by row indicate statistically significant differences ( $p < 0.05$ ). Last column present the range (minimum; maximum) of worm burden for each FAMACHA<sup>®</sup> score and the number of records in brackets [ ].

**Table 4.** Least square means (s.e: standard error) from ANOVA analysis and number of records in brackets [ ] of faecal worm egg count (FEC), FEC logarithmically transformed (LnFEC), packed cell volume (PCV), haemoglobin (Hb) and red blood cell count (RBC) for the four scores of Multistix<sup>®</sup> test under experimental *H. contortus* infection (10.000 L3).

Multistix <sup>®</sup>	LnFEC (Ln epg)	FEC (epg)	PCV (%)	Hb (g/dl)	RBC (cells $\times 10^5$ )	Worm burden
1	4.61 <sup>a</sup> (0.12) [96]	0 <sup>a</sup> (229) [96]	29.74 <sup>a</sup> (0.94) [66]	8.03 <sup>a</sup> (0.36) [36]	8.93 <sup>a</sup> (0.38) [36]	-
2	5.95 <sup>b</sup> (0.14) [68]	1374 <sup>b</sup> (272) [68]	23.99 <sup>b</sup> (0.94) [67]	6.87 <sup>b</sup> (0.32) [46]	7.43 <sup>b</sup> (0.34) [46]	1980; 6110 [3]
3	5.96 <sup>b</sup> (0.15) [63]	1359 <sup>b</sup> (283) [63]	21.47 <sup>b</sup> (0.97) [62]	5.99 <sup>b</sup> (0.39) [30]	6.64 <sup>b</sup> (0.42) [30]	4800; 8950 [22]
4	6.92 <sup>b</sup> (0.59) [5]	1775 <sup>ab</sup> (1121) [5]	22.00 <sup>ab</sup> (3.83) [5]	5.90 <sup>ab</sup> (1.22) [4]	6.25 <sup>ab</sup> (1.33) [4]	5090; 8710 [4]

\*Different superscript letters by row indicate statistically significant differences ( $p < 0.05$ ). Last column present the range (minimum; maximum) of worm burden for each Multistix<sup>®</sup> score and the number of records in brackets [ ].

infection (0.94 vs 0.84). Conversely, the rest of the correlations estimated were lower. The correlations between FEC and LnFEC with PCV and FAMACHA<sup>®</sup> were of moderate magnitude. The Multistix<sup>®</sup> test had a low correlation with FEC and LnFEC, while the correlation of this test with PCV and FAMACHA<sup>®</sup> did not differ statistically from zero ( $p > 0.05$ ).

The sex of the animals had a significant effect, with males having a higher FEC and a lower PCV than females. The values of the least squares means of FEC were 730 and 250 epg, and the values of PCV were 29.8% and 33.9% for males and females, respectively.

## Discussion

### Experimental challenge

The three tests were able to detect occult blood in the stool under experimental challenge. The principal limitation of FOB tests is that they are non-specific, as bleeding into the GI tract can be caused by several different pathologies. Additionally, the changes in Multistix<sup>®</sup> scores observed in some animals (seven lambs with score 3 that became 2 in the next

**Table 5.** Pearson correlation coefficients\* (n; 95% confidence interval) between faecal worm egg count (FEC), FEC logarithmically transformed (LnFEC), FAMACHA<sup>®</sup>, Multistix<sup>®</sup>, packed cell volume (PCV), haemoglobin (Hb) and red blood cell count (RBC) under experimental *H. contortus* infection (10.000 L3).

Trait	FEC	PCV	Hb	RBC	FAMACHA <sup>®</sup>	Multistix <sup>®</sup>
LnFEC	0.84 (272; 0.80, 0.87)	-0.71 (200; -0.63,-0.77)	-0.75 (116; -0.66, -0.82)	-0.75 (116; -0.66, 0.82)	0.63 (269; 0.56, 0.70)	0.46 (232; 0.35, 0.55)
FEC	-	-0.52 (200; -0.41,-0.61)	-0.63 (116; -0.51,-0.73)	-0.61 (116; -0.49, 0.72)	0.50 (269; 0.40, 0.58)	0.25 (232; 0.13, 0.37)
PCV	-	-	0.96 (116 0.94, 0.97)	0.98 (116; 0.97, 0.98)	-0.70 (200; -0.62,-0.76)	-0.37 (200; -0.24,-0.48)
Hb	-	-	-	0.94 (116; 0.91, 0.96)	-0.73 (116; -0.63,-0.80)	-0.31 (116; -0.13,-0.46)
RBC	-	-	-	-	-0.70 (116; -0.59,-0.78)	-0.32 (116; -0.15,-0.47)
FAMACHA <sup>®</sup>	-	-	-	-	-	0.55 (232; 0.46, 0.64)

\*Note: all the correlations were statistically different from zero ( $p < 0.001$ ).

**Table 6.** Pearson correlation coefficients (95% confidence interval) between faecal worm egg count (FEC), FEC logarithmically transformed (LnFEC), packed cell volume (PCV), FAMACHA<sup>®</sup> and Multistix<sup>®</sup> under field infection.

Trait	FEC	PCV	FAMACHA <sup>®</sup>	Multistix <sup>®</sup>
LnFEC	0.94*** (291; 0.93, 0.95)	-0.41*** (284; -0.31,-0.51)	0.22** (287; 0.11, 0.33)	0.13* (272; 0.01, 0.25)
FEC	-	-0.37*** (284; -0.27,-0.47)	0.19** (287; 0.08, 0.30)	0.14* (272; 0.02, 0.25)
PCV	-	-	-0.38*** (302; -0.28,-0.47)	0.02 <sup>ns</sup> (283; -0.09, 0.14)
FAMACHA <sup>®</sup>	-	-	-	-0.05 <sup>ns</sup> (286; -0.16, 0.07)

\*\*\* $p < 0.0001$ ; \*\* $p < 0.01$ ; \* $p < 0.05$ ; ns: not significant.

measurement) might be explained by the sensitivity of this test and the subjectivity to interpret the results. Colditz and Le Jambre (2008) studied the capacity of Hemastix<sup>®</sup> test (Bayer, Australia) to determine the severity of *H. contortus* infection in sheep at pasture, and they also found that the reagent sticks were able to detect blood in faeces. However, Wakid (2010) did not find a significant association between positive guaiac FOB test and infection with GI parasites in humans.

A FAMACHA<sup>®</sup> score of 3 was considered to be indicative of anaemia, which is in agreement with the studies of Kaplan *et al.* (2004) and Santos Sotomaior *et al.* (2012). In addition, Kaplan *et al.* (2004) found that FAMACHA<sup>®</sup> score of 3, 4 and 5 or 4 and 5 were considered anaemic and PCV values were considered anaemic if  $\leq 19\%$  or  $\leq 15\%$ , respectively.

For Multistix<sup>®</sup> test, only score 1 was statistically different from scores 2 and 3 for PCV. In contrast with this result, Colditz and Le Jambre (2008) observed that haematocrit did not differ significantly for Hemastix<sup>®</sup> from score 1 to 2.5 and then decreased significantly with subsequent increments in FOB score. These authors concluded that a

decrease in the haematocrit as indicative of anaemia was evident at FOB score 3 and above. Moreover, Multistix<sup>®</sup> gave negative results in most animals when Hb values were under 9 g/dl. This observation can be explained by the sensitivity of the test itself, since blood loss would not be detected before day 11 post-infection. This is supported by the fact that after day 11, there were no more false negative results. The haematological parameters decreased to sub-normal values since day 18 post-infection. According to Colditz and Le Jambre (2008), in the early stages of *H. contortus* infection there is little impact of blood loss due to parasitism on erythrocyte parameters, therefore anaemia can be considered to be a lagging indicator of the severity of *H. contortus* infection. The correlations between FEC, LnFEC, PCV, Hb and FAMACHA<sup>®</sup> estimated in the present study, are in agreement with previous findings (e.g. Bisset *et al.*, 2001; Kaplan *et al.*, 2004; Vanimisetti *et al.*, 2004). The correlation observed between worm burden and FEC was slightly lower than the reported in the literature, being in the range of 0.74 to 0.91 (e.g. McKenna, 1981; Bisset *et al.*, 1996; Pandey, 1999).

### Field infection

Larvae culture results confirmed the presence of *Haemonchus* spp., in agreement with those reported in Uruguay (Nari *et al.*, 1977; Castells, 2009). Despite the high percentage of *Haemonchus* spp., due to probably the high biotic potential of this parasite, worm burden of this genus could be low, explaining the results that most samples were negative to Multistix® and Hexagon test.

According to the protocol followed in the field infection sampling, probably most of the animals were in an early stage of parasitism and with low parasite burden (FEC mean value of 500 epg). These observations were supported by the PCV mean of 32.1% and the low percentage in samples with sub-normal values (6.8%), and that from 309 FAMACHA® records, 39.5% had score 1, 45.6% score 2 and 14.9% score 3. Therefore, animals could have been recently infected thus, as it was discussed earlier, in the early stages of *H. contortus* infection there is little impact of blood loss on erythrocyte parameters.

The sex of the lambs had a significant effect, which is in agreement with a great number of studies that reported that males are more susceptible to GI parasites than females (Barger, 1993; Pandey, 1999).

In conclusion, FEC and the haematological parameters decreased to sub-normal values on day 18 post-infection. However, FOB tests were able to detect *H. contortus* infection earlier (from day 11) under experimental challenge and with high parasite burden. Nevertheless, when animals were sampled under natural mixed-parasite infection, these tests were unable to detect occult blood in faeces in most samples. It could be due to a low worm burden of this genus and/or because animals had been recently infected. This is supported by the observation that FEC mean was 500 epg, 93.2% of PCV records had normal values and 85.1% of the individuals had FAMACHA® scores 1 or 2. FAMACHA® and PCV demonstrated to be good indicators of haemonchosis, being moderately to highly correlated with FEC. Finally, FOB tests could be useful for early diagnosis of haemonchosis under high *Haemonchus* spp. parasite challenge. However, they are not recommended for routine diagnosis since most cases under natural challenge are composed by mixed-parasite species and *Haemonchus* spp. burden might not be high enough for these tests to detect occult blood in faeces.

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### Conflict of interest

The author declares that there is no conflict of interest.

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