Validation of the FAMACHA© method for detecting anemia in Cuban Pelibuey sheep

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ABSTRACT

During a period of three years, the reliability of the FAMACHA© method in the identification of anemic animals as a result of parasite infestation by *Haemonchus* spp. was studied in a Pelibuey sheep flock of 75 dams. With a monthly frequency, the fecal egg count (FEC) and the hematocrit (PCV) were evaluated; and the body condition (BC) and color of the ocular mucosa (COM) were monitored through the FAMACHA© color chart. In addition, the sensitivity, specificity and predictive value for positive and negative animals were determined; as well as the agreement index (*Kappa*) to establish the agreement between COM and PCV. The correlation among the variables was also studied. It was proven that there is moderate agreement (0,25-0,31) between the identification of anemic animals and the color of the ocular mucosa through the FAMACHA© chart. The reliability indicators showed discreet values and were related to the hematocrit value selected as gold standard (PCV ≤ 17 %). An inverse ratio was found ($r = -0,437$, $p < 0,01$) between the FEC and PCV. The FAMACHA© method is concluded to constitute a useful and practical tool for the identification of anemic sheep dams as a consequence of a high infestation by *Haemonchus* spp.

Key words: anemia, FAMACHA©, *Haemonchus*, sheep

INTRODUCTION

In Cuba, for several decades, parasite control has consisted in treating the animals according to the subjective criteria of parasite infestation. In general, the flocks are integrally treated based on clinical signs of infestation or at prefixed times, which are possibly related to the moments of higher risk. This situation has led to the emergence of resistance, in many places, to the most widely used drugs (Arece *et al*., 2004).

In the current context the application of selective treatments is among the most accepted principles, because it has been proven that barely 30 % of the flock has more than 90 % of the parasite rate (Barger, 1985). This explains the importance of the adequate and accurate identification of the animals that have a higher parasite infestation rate, especially under field conditions.

The FAMACHA© method –originally developed in South Africa– is based on the identification of the animals with clinical anemia, through the inspection of the ocular mucosa (Bath *et al*., 1996). It relies on the presence of *Haemonchus* spp. as basic principle, situation which has been widely documented in Cuba (García *et al*., 1999; Arece *et al*., 2007), and it constitutes the parasite with the highest prevalence and incidence in the tropic and in the country.

This method has been validated under different conditions in several countries; however, to extend its productive practice it is required to prove its efficacy in the identification of anemic animals as a consequence of parasite infestation by *Haemonchus* spp., which constituted the objective of this study.

MATERIALS AND METHODS

The study was conducted in a Pelibuey sheep flock from the research-production area of the Pasture and Forage Research Station Indio Hatuey, Matanzas, Cuba. This flock was subject, during three years, to a parasite control strategy based on selective treatments, related to the color of the ocular mucosa –FAMACHA© method– (Bath *et al*., 1996).

During this period, with a monthly frequency, the color of the ocular mucosa (COM) of each dam was examined, and it was classified into each of the five categories of the FAMACHA© color chart

(Van Wyk *et al*., 2006), in which 1 corresponds to an animal with intense red coloring, and 5, to one with pale mucosae. The body condition (BC) was also monitored (Russel *et al*., 1969) and blood was extracted through puncture of the jugular vein, in order to evaluate microhematocrits (PCV) by centrifugation (Hansen and Perry, 1994). Additionally, the feces were directly extracted from the rectum of each animal for determining the strongyle fecal egg count (FEC) as criterion of parasite infestation –through the modified McMaster technique (Arece *et al*., 2002)–, with a sensitivity of 50 eggs per gram of feces (EPG) and the performance of fecal cultures (Roberts and O'Sullivan, 1952) and the identification of the nematode species present.

The animals grazed in an association of shrubby legumes (*Leucaena leucocephala, Albizia lebbeck* and *Gliricidia sepium*) and grasses (*Panicum maximum* and *Dichanthium-Bothriochloa* complex), with a global stocking rate of 25 animals/ha and a rotation of 12 paddocks. In addition, they received an additional diet consisting in citrus fruit silage, *L. leucocephala* foliage and fresh citrus fruit pulp, at three moments of the reproductive cycle.

The information obtained from the studied variables during the three years was used to validate the reliability of the method in anemia detection. The agreement between PCV and the estimations made with the color chart was determined using the agreement index (*Kappa*); through this index the magnitude of the agreement or relationship between two tests (for example: the hematocrit of an animal and the assigned values according to the FAMACHA© color chart) was established. The conventional divisions for the interpretation of *Kappa* indicate that it is insignificant if lower than 0,2; moderate, if it is between 0,2 and 0,4; acceptable, if it is within 0,4-0,6; good; if it is between 0,6 and 0,8; and excellent, if it is higher than 0,8 (Mahieu *et al*., 2007). This was calculated for different hematocrit levels considered anemic, because the indicated anemia value in Cuban Pelibuey sheep is not accurately known; for such reason, four values were used, lower than: 17, 19, 21 and 23 %.

Likewise, the sensitivity [(true positives + false negatives)/true positives x 100)], specificity [(true negatives + false positives)/true negatives x 100)] and the predictive values of a positive and a negative, were calculated (Vatta *et al*., 2001). The animal with a PCV lower than or equal to 17, 19, 21 and 23 % was considered a positive one; with regards to the FAMACHA© color chart, categories 4 or 5 were taken into consideration. On the other hand, a negative animal should show a PCV higher than the previously fixed values, and the color of the mucosa should fluctuate between categories 1 and 3 of the color chart.

The determination of the above-mentioned epizootiological indicators was performed by using the software WinEpiscope 2.0 for Microsoft Windows®. Besides, the non-linear correlations (Spearman's correlation coefficient) among the BC, COM, FEC and PCV were calculated with the statistical pack SPSS® version 18.0 for Microsoft Windows®.

RESULTS AND DISCUSSION

The management of haemonchosis in a flock using the FAMACHA© color chart depends on the accurate identification and adequate treatment of the highest egg-expelling animals, as well as on the fact that the others remain untreated. Thus the general resilience of the flock would increase and the parasite population in refuge would be kept at an adequate level.

Table 1 shows the reliability of the FAMACHA© color chart in the detection of anemic dams. The best results regarding the agreement index were obtained when all anemic animals with PCV lower than 23 % were considered (*Kappa* = 0,31). These values are similar to the ones obtained in

Table 1. Reliability indicators of the diagnostic test.

Indicator	PCV value			
	< 17 %	< 19 %	< 21 %	< 23 %
Kappa	0,30	0,25	0,29	0,31
Sensitivity	63,51	39,41	52,26	49,59
Specificity	90,96	91,58	84,79	87,18
Predictive value –	98,23	95,12	92,40	87,75
Predictive value +	23,98	26,60	33,42	48,15

goats, in the Eastern Caribbean (Guadeloupe) (Mahieu *et al*., 2007). From the practical point of view, this value indicates that the identification of anemic animals, from the color of the ocular mucosa, is possible with a moderate reliability level.

On the other hand, sensitivity (capacity of the method to detect the really anemic animals) showed moderate values and specificity (capacity to detect the non-anemic animals) had results over 84 %. This depended on the hematocrit value selected for the evaluation of the diagnostic test; in this sense, a better adjustment was found when the animal with hematocrit lower than 17 % was considered anemic.

In this type of essay, high sensitivity is more important than high specificity (Scheuerle *et al*., 2010). This implies that if an animal is classified as false positive and, thus, it is treated, it would only have repercussion on an unnecessary expense of the antiparasitic drug. However, if an animal considered false negative is not treated it could die –in the worst case–, depending on the frequency of animal checking.

Arece (2007), under similar conditions as the ones in this study, obtained a sensitivity of 75 % and a specificity of 100 %. Probably, such results are related to the size of the sample used, which could have biased their reach.

In the Matanzas province, in studies with dairy goats, a sensitivity of 95 % was found. However, the specificity of the method was lower (62,4 %) than the one found in this research.

If the results obtained in other countries are analyzed, the reliability values are observed to vary from 19 to 85 % for sensitivity, and from 52 to 100 % for specificity (Vatta et al., 2001; Kaplan *et al*., 2004; Burke *et al*., 2007; Mahieu *et al*., 2007). According to Reynecke *et al*. (2011) the use of the color chart for the selective treatment of the animals –taking into consideration the reliability indicators– constitutes a practical tool for farmers, without needing the routine intervention of laboratories.

Table 2 shows the statistically significant correlation between the FEC and PCV, due to the prevailing presence of *Haemonchus* spp., of which its high pathogenicity, virulence and hematophagous capacity are known (Hansen and Perry, 1994). In addition, the negative and significant correlation between the COM and BC of the dams is observed, which indicates that the body status of the dam could constitute an element for selective deworming, when there are doubts in the color of the ocular mucosa. This physiopathological variable has been significantly correlated with the COM, the FAMACHA© categories, the live weight and the hematocrit, which reveals the possibility of its use as deworming category in the presence of a haemonchosis (Bisset *et al*., 2001). Nevertheless, Bath and Van Wyk (2009) state that it must be treated with caution within a sustainable, holistic and integral program for parasite control.

The results of the relationship of the hematocrit and the categories according to the FAMACHA© color chart are shown in figure 1. Categories 4 and 5 were assigned only to 3,35 % of the cases, which indicates a low rate of antiparasitic treatments, according to their selective method; and confirms the theory that the lowest portion of a flock has the highest parasite rates.

On the other hand, it was found that only 2,3 % of the cases showed a hematocrit equal to or lower than 15; while in 5,4; 9,5; 15,2 and 24,9 % this indicator was lower than or equal to 17, 19, 21 and 23, respectively. During the three years, 96,6 % were classified in categories 1, 2 and 3, which corresponds to non-anemic dams. The use of this method to identify the most susceptible animals in the flock could contribute to the implementation of integral

Table 2. Correlation coefficient between BC, COM, FEC and PCV.

	BC	COM	FEC	PCV
BC		-0,469** (2183)	-0,274** (1875)	0,389** (1906)
COM	-0,469** (2183)		0,290** (1874)	-0,413** (1906)
FEC	-0,274** (1875)	0,290** (1874)		-0,437** (1647)
PCV	0,389** (1906)	-0,413(**) (1906)	-0,437** (1647)	

** Significant correlation for $p < 0,01$ (*n*): sample size

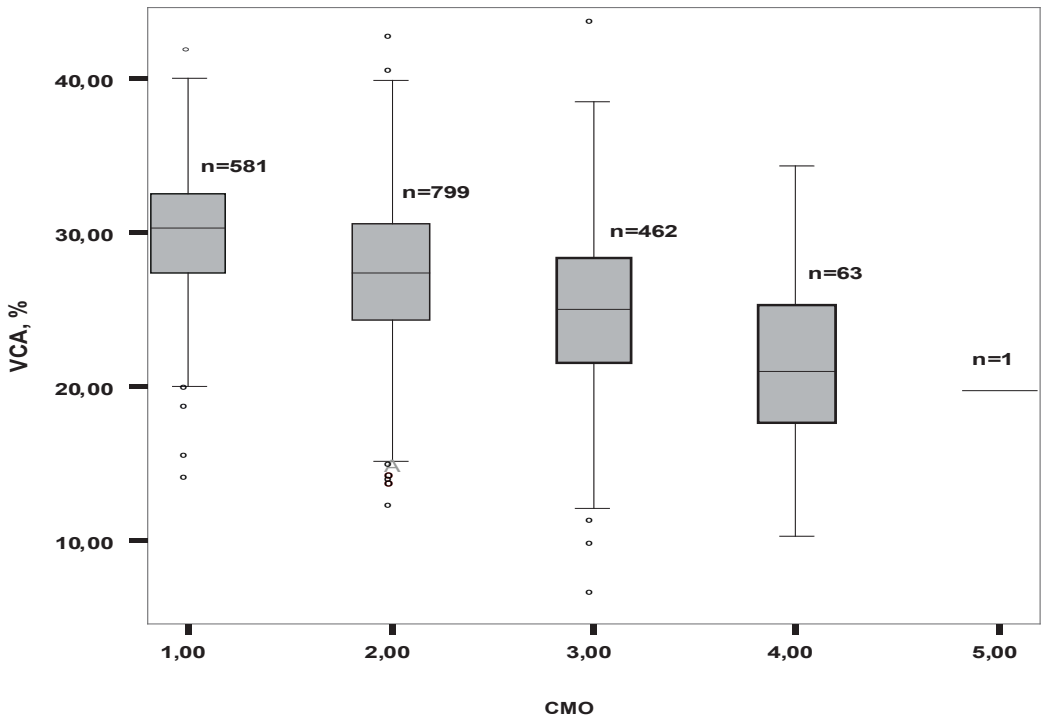

Figure 1. Relationship between the values assigned through the FAMACHA© color chart and the corresponding hematocrit.

strategies for parasite control, with emphasis on the selection of those resilient or resistant to gastrointestinal parasitism (Bisset *et al*., 2001; Riley *et al*., 2009; Vilela *et al*., 2012).

It is concluded that the FAMACHA© method constitutes a viable tool for anemia detection in Pelibuey sheep, and that it could be applied in Cuba within an integral parasite control program.

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