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Evaluation of strategic and selective anthelmintic treatments on Pelibuey ewes in Cuba¤

Evaluación de tratamientos antihelmínticos estratégicos y selectivos en ovejas Pelibuey en Cuba

Avaliação de tratamentos anti-helmínticos estratégicos e seletivos em ovelhas Pelibuey em Cuba

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Summary

Background: gastrointestinal parasitism is an important limitation for sheep production in Cuba. This situation is worsened by the lack of an effective parasite control strategy. **Objective:** to assess whether selective and strategic treatments could help achieve a level of control of nematodes similar to systematic drenchings. **Methods:** an experimental group of 73 Pelibuey ewes was divided into four groups: 1) the Epizoo group (Epizootiological drenching schedule) was dewormed at lambing in the middle of the dry season and at weaning; 2) the FAMACHA© group was treated according to the color of ocular mucosa as indicative of anemia (Categories 4 and 5 of the FAMACHA© color chart); 3) the Chemical group was drenched every three months; and 4) the control group, in which animals remained untreated unless fecal egg count (FEC) was higher than 1500 eggs per gram of feces or the packed cell volume (PCV) was lower than 15%. FECs, body scorings (BCS), hematocrits and color of ocular mucosa (COM) were evaluated monthly. **Results:** the control group showed the highest FEC and the lowest PCV. We also observed similar levels of FEC and body condition (BCS) in the Epizoo, FAMACHA© and Chemical groups. The PCV in FAMACHA© group was the highest, while no differences were found between the other two groups. Hematocrit had a strong negative correlation (-0.41) with FEC. The FAMACHA© method allowed a dramatic decrease in anthelmintic use. **Conclusions:** the obtained results suggest that FAMACHA© method could be incorporated as part of an effective parasite control strategy in sheep.

Keywords: *anemia, mucous membranes, Haemonchus spp., sheep, deworming.*

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Resumen

Antecedentes: el parasitismo gastrointestinal constituye una de las principales limitantes para producción de ovejas en Cuba. Esta situación es mucho más grave porque no existe un programa adecuado de control parasitario. **Objetivo:** evaluar si el uso de un tratamiento antihelmíntico estratégico o selectivo logra reducir los niveles de infestación parasitaria comparado con un sistema de tratamientos químicos sistemáticos. **Métodos:** se utilizó un rebaño experimental de 73 reproductoras Pelibuey que se dividió aleatoriamente en cuatro grupos: 1) El grupo epizootiológico (Epizoo) se trató al parto, a mediados del periodo seco y al destete, 2) el grupo FAMACHA© se desparasitó en función de la coloración de la mucosa ocular indicativa de anemia (categorías 4 y 5 de la carta de colores FAMACHA©), 3) el grupo Químico: se desparasitó cada tres meses, y 4) grupo control: no recibió tratamiento mientras su hematocrito (PCV) fuera inferior a 15% o la carga parasitaria (FEC) no superara los 1500 huevos por gramo de heces. Con frecuencia mensual se determinó a cada animal el conteo fecal de huevos (FEC), la condición corporal (BCS), el hematocrito (PCV) y la coloración de la mucosa ocular (COM). **Resultados:** se observaron valores similares para la BCS y el FEC en los grupos Epizoo, FAMACHA© y Químico. El PCV presentó los valores más altos en el grupo FAMACHA© y el grupo Control presentó los más bajos; no se apreciaron diferencias significativas entre los grupos Químico y Epizoo. El grupo control presentó los mayores FEC y los más bajos PCV. El hematocrito tuvo una relación fuerte y negativa (-0,41) con el FEC. El método FAMACHA© propició una disminución en el uso de fármacos antihelmínticos. **Conclusiones**: los resultados obtenidos sugieren que el método FAMACHA© podría ser incorporado como parte de una estrategia efectiva de control parasitario en ovinos.

Palabras clave: *anemia, membranas mucosas*, *Haemonchus* *spp., ovinos, desparasitación.*

Resumo

Antecedentes: o parasitismo gastrointestinal é uma limitação para a produção de ovinos em Cuba. Esta situação é agravada pela falta de uma estratégia eficaz de controle parasitário. **Objetivo:** avaliar se os tratamentos seletivos podem ajudar a alcançar um nível de controle de nematóides semelhante a dois tratamentos sistemáticos. **Métodos:** um rebanho experimental de 73 ovelhas foi dividido em quatro grupos: 1) o grupo Epizoo (Esquema de tratamento epizootiológico) foi tratado no parto, no meio da estação seca e ao desmame, 2) o grupo FAMACHA© foi tratado de acordo com a cor da mucosa ocular (categorias 4 e 5 na cartela de cores FAMACHA©), 3) o grupo químico são vermifugados a cada três meses, e 4) o grupo controle não recebeu tratamento, enquanto seu hematócrito (PCV) fosse inferior a 15% ou a carga parasitária (FEC) não ultrapassasse 1.500 ovos por grama de fezes. A contagem de ovos por grama (FEC) de fezes, o escore da condição corporal (BCS), hematócrito (PCV) e a coloração da mucosa ocular (COM) foram avaliados mensalmente. **Resultados:** um nível similar de FEC e BCS foram observados nos grupos Epizoo, FAMACHA© y Químico. O PCV apresentou-se maior no grupo FAMACHA© e o grupo Controle teve os valores mais baixos; enquanto nenhuma diferença foi estimada entre os outros dois grupos. O grupo controle apresentou o maior FEC e o menor PVC. O hematócrito teve uma forte correlação negativa (-0,41) com o FEC. O método FAMACHA© permitiu uma diminuição drástica do uso de anti-helmíntico. **Conclusões:** os resultados sugerem que o método FAMACHA© pode ser incorporado como parte de uma estratégia para o controle de parasitas gastrintestinais em ovelhas.

Palavras chave: *anemia, membranas mucosas,* *Haemonchus* *spp., cordeiro, desparasitação.*

Introduction

Gastrointestinal parasitism is one of the most important natural factors affecting the sheep industry worldwide. Numerous studies have been aimed at finding alternative strategies to control gastrointestinal parasites, such as FAMACHA©, nematophagous fungi, plant secondary metabolites, among others (Waller *et al*., 2001; Burke *et al*., 2004; Kaplan *et al*., 2004; Rojas *et al*., 2006; Leask *et al*., 2012).

Anthelmintic control plans in Cuba are focused only on an excessive use of anthelmintics, leading to the development of anthelmintic resistance (Arece *et al*., 2004). Preference for chemical control strategies has become a common practice based on the belief that it is the fastest method for controlling infection. When confronted with the need to keep infection under control, producers tend to choose the chemical strategy over any other alternative because of the rapid response to fecal egg count (FEC), thus reducing group mortality and improving weight gain.

In order to increase the overall efficacy of parasite control strategies for small ruminants, an epidemiological study was conducted in Matanzas province (Cuba). Several strategies to provide the best control result were tested, given the multiplicity of factors involved. This study demonstrated the prevalence of a predominant infection by *Haemonchus* spp. throughout the year, without seasonality (Arece, 2007). Under this circumstance, the FAMACHA© method revealed itself as a practical tool for detecting anemic animals (Arece *et al*., 2007) based on the relationship between packed cell volume (PCV) and FEC, as well as on the high sensitivity and specificity of the test, among other indicators. The FAMACHA method, developed in South Africa by Malan *et al.* (2001), is a simple system to categorize the anemic status of small ruminants based on the conjunctival mucosa color on a scale from 1 (optimal eye color, red) to 5 (pale eye color, white), especially when animals are predominantly infected by *H. contortus*. This study also showed well-defined epidemiological risk factors, such as parturition, age, ewe reproductive status, and animal category among others, providing groundwork to design a tailored drenching control method (Arece, 2007). Despite these obvious results, there is still a need to evaluate the efficacy of different strategies, and even the possibility of integrating elements of some of them. Thus, the objective of the present study was to evaluate the efficacy of three other parasite control strategies in a silvopastoral system.

Materials and methods

Experimental site and management

The trial was conducted in the research-production facility of small ruminants at the Estación Experimental de Pastos y Forrajes "Indio Hatuey" located in the Perico municipality, central zone of the Matanzas Province, at 22º 48’ 7’’ N and 79º 32’ 2’’ W, located 19.9 meters above sea level. The grazing area was based on a silvopastoral system with predominance of *Leucaena leucocephala* and *Panicum maximum*, combining trees and grasses. The global stocking rate was 19 ewes and their lambs per hectare, allowing the animals to graze in a rotational system for six hours daily in 12 paddocks. The reproductive mating periods were organized by campaigns: late May and late November.

The ewes received additional supplementation, based on mixed citrus silage, three times: at mating, at last trimester of pregnancy, and during the first 30 days after lambing, in coincidence with the moments of highest metabolic demand (López *et al*., 2008). Water and mineral salt were supplied *ad libitum*.

Animals, treatments, and experimental procedure

Seventy-three Pelibuey ewes (35 ± 2.5 kg body weight, BW; 4 years old) were randomly assigned to four groups: Chemical, Epizoo, FAMACHA© and Control. All animals were kept in the same group and management system. The Chemical group (n = 17) was treated with Levamisol 10% (Levamisole clorhidrate at 7.5 mg/kg BW, Labiofam, Cuba) or LABIOMEC® (Ivermectine at 0.22 mg/kg BW, Labiofam, Cuba) every three months. The Epizoo group (n = 19) was treated following the design of a previous strongyle epidemiological study conducted in this group (Arece, 2005) and by means of the FAMACHA© selective treatment strategy. All ewes in this group were targeted and drenched on three occasions: at parturition, in the middle of the dry season and at weaning. The rest of the year ewes were selectively treated according to the FAMACHA© method (animals in categories 4 and 5 were drenched). In the FAMACHA© group (n = 19) ewes were selectively treated according to the FAMACHA© method, this is, animals with categories 4 and 5 in the FAMACHA© chart were drenched. Finally, the Control group (n = 18) was left untreated, unless hematocrit was less than 15% or FEC was greater than 1500 eggs per gram of feces (EPG).

Ethical considerations

The Indio Hatuey Animal Care and Use Committee and the Scientific Council of the Indio Hatuey Experimental Station approved animal procedures (Minute No. 20, February 26, 2006) in accordance with the Cuban Animal and Plant Protection Society (ANIPLANT).

Samples and measurements

Animals in all groups were sampled for FEC on a monthly basis using the McMaster flotation technique (Arece *et al*., 2002). Feces were collected directly from the rectum of each animal. Additionally, coprocultures were prepared according to each experimental group and sampling date (Roberts and O´Sullivan, 1952) and the infective third-stage larvae were recovered and identified through a morphological key (Valle, 1975).

Blood samples were taken through jugular puncture in sample tubes containing EDTA for PCV determination (microcentrifugation at 12,000 rpm for 5 min), on a monthly basis. Body condition score (BCS) of each ewe was assessed in which score one (1) corresponded to an extremely emaciated animal on the verge of death, and five (5) represented the fattest animal (Russel *et al*., 1969). The BCS assessment was carried out by the same trained person each month. Finally, color of the ocular mucosa was examined monthly and classified into one of five categories according to the FAMACHA eye color chart: 1 = red, non anemic; 2 = red-pink, non anemic; 3 = pink, moderately anemic; 4 = pink-white, anemic and 5 = white, severely anemic (Bath *et al*., 2001).

Meteorological information

Data on daily rainfall and temperature during the experiment were collected. The monthly maximum and minimum temperatures were recorded at the nearest meteorological station (Indio Hatuey).

Data analysis

To compare treatment results we used a mixed model (SAS®, Proc. Mixed, SAS Institute Inc., Cary, NC, USA). for FEC and PCV variables, testing three covariance structures: auto-regressive, unstructured and compound symmetric. The structure selection procedure was based on the highest Akaike's (AIC) and Bayesian (BIC) information criteria (Littell *et al*., 2006).

The FEC values were previously transformed to their natural log to stabilize variances (Gauly and Erhardt, 2001). Differences between treatments were assessed through Duncan's test.

Additionally, data from the FAMACHA© group were analyzed independently. Spearman correlation coefficients were calculated to examine the relationship between eye scores, PCV and FEC. To better visualize results, box and whisker plots were drawn to represent the distribution of PCV and BCS in relation to FAMACHA© scores (Kaplan *et al*., 2004).

Results

Figure 1 describes the climatic conditions of the experimental period. The general trend was for the minimum mean temperature to decrease during the months when rainfall also decreased. Thus, the period from mid-May to October was warm and humid, referred to as the rainy season, while the rest of the year was considered the dry season. There are two well-defined weather periods in Cuba: rainy season (late May to October) and dry season (November to April). The accumulated rainfall during the rainy season represented 81% of the total rainfall recorded in the year.

Table 1 shows FEC distribution in the four groups. No differences ($p>0.01$) where detected between FAMACHA©, Epizoo and systematic chemical drench groups when comparing these treatment strategies. The control group showed the highest FEC ($p<0.01$) with a considerable egg count.

The average percentage of nematode L_3 larvae from the composite fecal cultures among all groups of animals revealed that *Haemonchus* spp. was the dominant species, representing between 88 and 100% of EPG throughout the entire study period. *Trichostrongylus colubriformis* and *Oesophagostomum columbianum* were also present with an annual average of 3.93 and 2.31% for each species, respectively.

The FEC dynamics analyzed in each ewe group showed a similar general trend independent from the drenching frequencies. The highest infection degree appeared in the control group followed by ewes under the selective drench group (Figure 2). The highest FEC were recorded during the dry season and an outbreak occurred during November in all groups. The control group was drenched twice during the experiment because of severe anemia (average PCV less than 15%) and high FEC (more than 4,000 EPG).

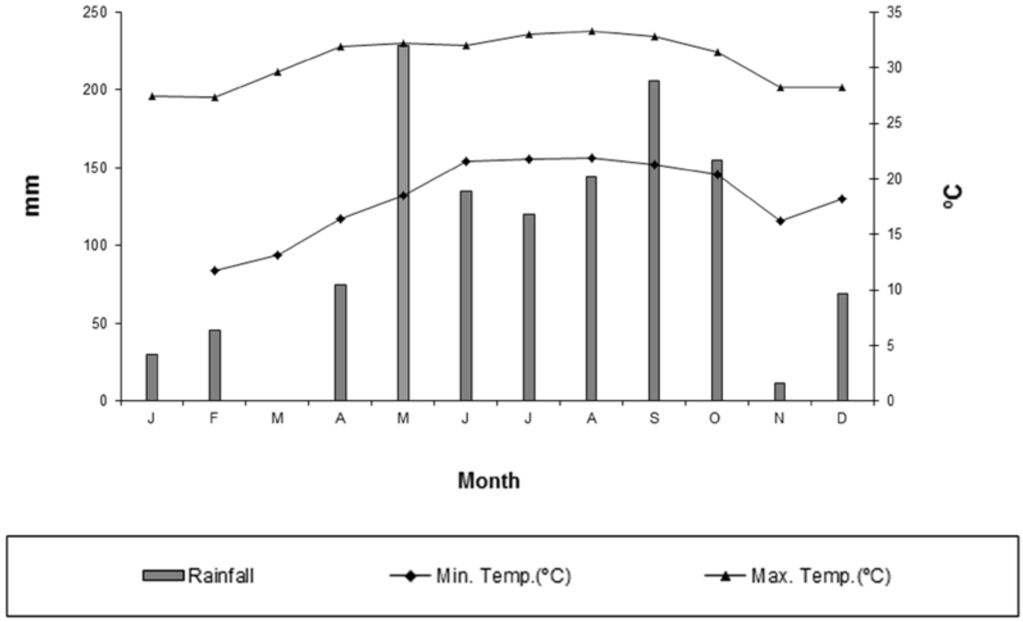

Figure 1. Metereological data at Indio Hatuey EEPF during the study period (year 2010).

Table 1. Mean and standard error (SE) of fecal egg counts (FEC), packed cell volume (PCV) and body condition score (BCS) for each experimental group.

Treatment	Mean (SE)		
	FEC	PCV	BCS
Chemical	421.03 (125.99)b	27.3 (0.39)b	3.17 (0.06)b
Epizoo	305.93 (59.05)b	27.86 (0.39)b	3.48 (0.08)a
Control	968.68 (171.51)a	27.34 (0.36)b	3.03 (0.05)b
FAMACHA©	478.5 (103.36)b	28.72 (0.35)a	3.2 (0.05)ab

[a,b] Means within a row with different superscripts are different (p<0.01).

The FAMACHA© ewes had the highest PCV values (p<0.01), while no differences were detected between the Epizoo and Chemical groups (p>0.05). The control group, as a result of high parasite infection, had the lowest PCV (Table 2, Figure 3). In general, PCV had a strong negative correlation with FEC (r = -0.41, p<0.01); that is, as PCV increases during the rainy season the mean FEC in each group decreases, and vice versa. All groups showed a similar trend during the experimental period, characterized by the highest PCV during the rainy season. PCV showed significant differences (p<0.01) between

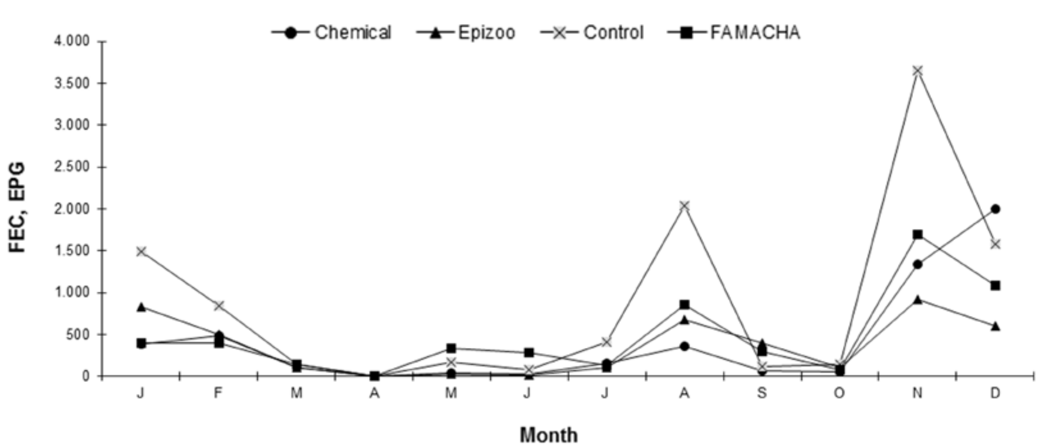

Figure 2. Fecal egg count (FEC) dynamics in experimental groups.

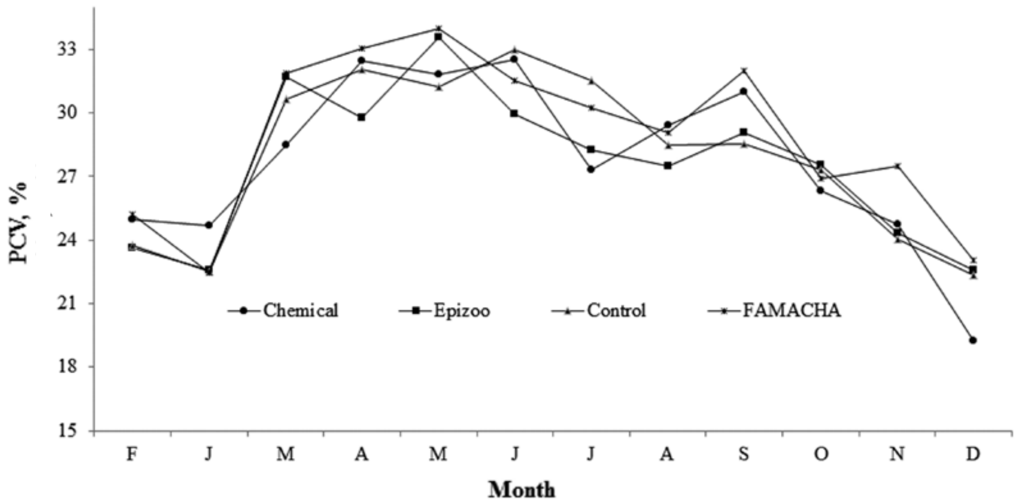

Figure 3. Dynamics of mean packed cell volume (PCV) of each experimental group.

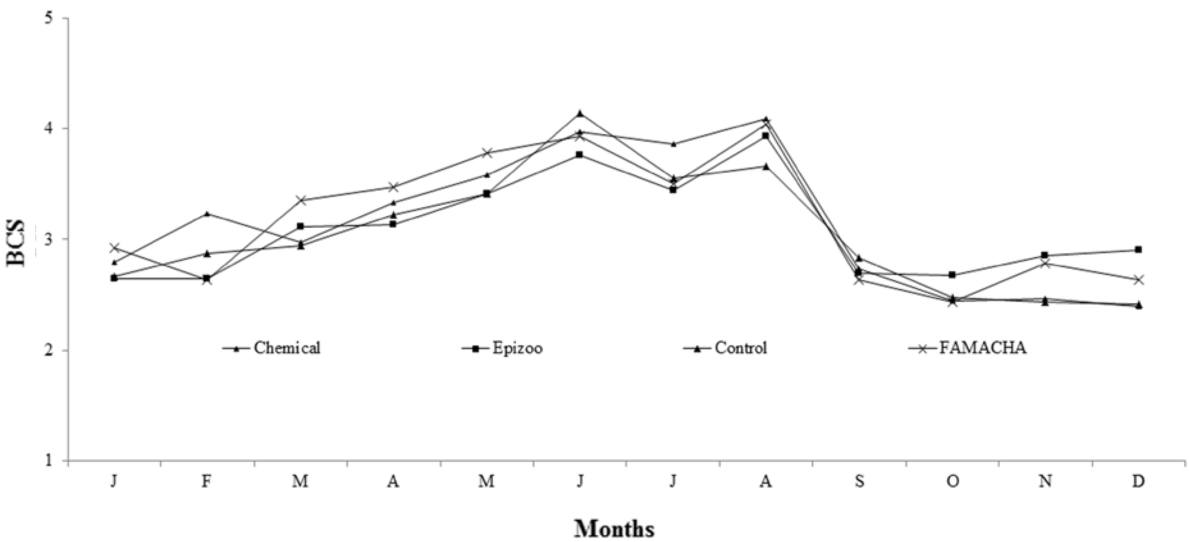

Figure 4. Dynamics of mean body condition score (BCS) of ewes in each experimental group.

Table 2. Mean and standard error (SE) of fecal egg counts (FEC, EPG), packed cell volume (PCV %) and body condition score (BCS) in the FAMACHA group.

FAMACHA© score	Mean (SE)		
	FEC	PCV	BCS
1	253 (62)	31.33 (0.55)	3.52 (0.05)
2	355 (96)	29.75 (0.51)	3.52 (0.04)
3	1,296 (559)	27.80 (1.04)	2.93 (0.04)
4	5,700 (3,679)	26.50 (0.21)	2.69 (0.02)
5	5,000	21.99	2.5

the dry and rainy seasons, with values of 25.5 ± 0.26 and 30.0 ± 0.20, respectively. PCV was negatively correlated with ocular mucosa color ($r = -0.35$, $p<0.01$) and FEC ($r = -0.32$, $p<0.01$) in the FAMACHA group.

No important changes in BCS were assessed between groups during the year (Table 1). All ewes showed acceptable average BCS (between 2.8 and 4) throughout the year. The Epizoo group showed the highest BCS followed by the FAMACHA© group.

BCS was significantly different according to season ($p<0.01$) with values of 2.7 ± 0.72 and 3.5 ± 0.76 for the dry and rainy season, respectively. As for PCV, the animals had a decrease on body score during the dry season (Figure 4).

The number of anthelmintic treatments within each experimental group differed highly. The control group required two non-programmed treatments, while the FAMACHA© group received five treatments in a year (0.02 treatment per ewe/year), all of them during the dry season. The chemical and Epizoo groups required more than three drenches per animal.

Ewes under the FAMACHA© strategy showed a progressive PCV decrease as the ocular mucosa became white in color. An inverse effect was observed for the FEC and body condition score (Table 2).

Discussion

Haemonchus spp. was the most prevalent gastrointestinal nematode parasite affecting the sheep in the four experimental groups, representing more than the 88% of FEC. A previously conducted study in this group revealed the predominance of this genus throughout the year without evident seasonality. Ewes were also infected by *T. colubriformis* and *O. columbianum*. Occasionally, infections by *Strongyloides* spp. were also observed. Thus, the parasitic infection structure remained similar in the four groups. Apart from the anthelmintic treatment strategy, the genus *Haemonchus* spp. continued as predominant in parasite infections, which is the main health problem of sheep in the region (Arece *et al*., 2007). The scheme of selective treatments against *Haemonchus* spp. prevented other species from occupying its ecological niche.

The high prevalence of *Haemonchus* infection in this group strongly suggested that FAMACHA© methodology is a proper tool for parasite control (Van Wyk *et al*., 2006). The use of this selective drenching strategy during one year did not lead to differences in FEC in comparison with the Chemical group.

FEC concentrations during the dry season should be analyzed carefully as drought is an important factor that enhances anthelmintic resistance (Papadopoulos *et al*., 2001). We suggest that a method that enhances group resilience, such as supplementation (Torres-Acosta *et al*., 2004; Houdijk *et al*., 2012), would be beneficial mainly because this epizootiological pattern is a result of a poor nutritional status (Arece *et al*., 2007).

Initial studies evaluating FAMACHA© under the conditions of the present trial resulted in sensitivity higher than 75% when animals with PCV lower than 19% and FAMACHA© color chart values between 4 and 5 were considered anemic (Arece *et al*., 2007).

Infection levels did not differ for animals with three antiparasitic treatments. Their average annual fecal counts did not exceed 500 EPG. The dynamics of this FEC showed similar patterns in all cases, indicating that parasitism performance is independent from the strategies adopted because their effects are shown in the magnitude of infection. Parasitism is a complex phenomenon whose manifestation responds to multispecific situations, such as immunological and nutritional status of the host and its reproductive stage, among others (Barger, 1999; Coop and Kyriazakis, 1999).

A *Haemonchus* outbreak related to periparturient ewes was recorded in August. FEC rose because most lambings occurred during that month. This well documented phenomenon has been explained by an immunity depression around parturition, favoring ovoposition of female parasites (Barger, 1993).

Body condition, besides indicating the nutritional status of the group (Hernández-Russo *et al*., 1999), is closely correlated with *Haemonchus* ssp. infection (Hoste and Chartier, 1993). BCS variations were related to bioproductive and ecological events. The poorest body condition was related to the highest infection levels in all groups. Group events, namely, estrus, pregnancy, parturitions, periparturient rise, etc., are directly related with feed availability (pastures and forage), which, in turn, is conditioned by season because feeding modulates animal reproduction (Rosa and Bryant, 2003).

Pelibuey sheep have a cyclical reproductive performance throughout the year (Perón *et al*., 1991).

Nevertheless, due to the presence of well-defined dry and rainy seasons, groups are forced into a mating strategy to concentrate parturitions, increasing the risk of disseminating the infection around grazing areas during peripartum. Implementing reproduction campaigns, on the other hand, facilitates offspring deworming and ewe management.

The FAMACHA© system seems appropriate not only for indirectly monitoring haemonchosis, but also for controlling it. We suggest a shorter monitoring interval during the dry season and lambing period when the epizootiological risk is increased (Arece *et al*., 2007). Weekly inspections should be conducted in ewes with medium to poor body condition, recently lambed ewes or when diarrhea is observed, which can be easily identified (Mahieu *et al*., 2007; Bath and Van Wyk, 2009). Such animals are much more likely to require drenching than those in good condition, as suggested by the positive relationship between BCS and FAMACHA© score.

As expected, FAMACHA© allowed for a drastic reduction in anthelmintics. This treatment not only impacts results in terms of economic benefits by reducing the number of anthelmintic treatments, but also in terms of ecological benefits by leaving part of the group untreated and decreasing anthelmintic resistance.

In our study, mean FEC and standard deviation for FAMACHA© scores 1, 2 and 3 was 482.5 and 1,347.3 EPG, respectively. This means that a considerable quantity of parasite eggs is dropped to the pasture, increasing the *refugia* population, which could probably survive long periods due to favorable climatic conditions. Therefore, it is reasonable to assume that it reduces selection pressure for anthelmintic resistance due to a dilution of the resistant population, particularly in rotational systems. Recently, the role of exploiting *refugia* for restoring anthelmintic activity of resistant drugs was demonstrated under field conditions (Sissay *et al*., 2006), which is an additional advantage of the selective treatment strategy.

Integration of FAMACHA© and scheduled drenching will be soon evaluated as we consider that their combination would reduce the epizootiological risk emerging from the periparturient rise and FEC increase occurring during the dry season (Arece *et al*., 2007).

The use of FAMACHA© and targeted treatments reached health indicators similar to a system of repeated anthelmintic treatments, leading to a reduction antiparasitic treatments. This method constitutes a tool for designing an integral strategy of sheep parasite control in Cuba.

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Conflicts of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

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