



Research paper

Sensitivity and specificity of the FAMACHA© system in growing lambs

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ABSTRACT

The FAMACHA© system is used for targeted selective treatment (TST) of small ruminants for the control of haemonchosis. The aim of this study was to evaluate the sensitivity and specificity of this system in growing lambs. Between 2015 and 2016, lambs aged 60–210 days on two sheep farms were evaluated at 15-day intervals using the FAMACHA© system (F1–F5), with analysis of hematocrit (Ht), counting of eggs per gram of feces, and larval culture. The sensitivity and specificity of the system were calculated according to two criteria: criterion 1, lambs classified as F4 or F5 were considered anemic (positive test) and those classified as F1, F2, or F3 were considered not anemic (negative test); and criterion 2, lambs classified as F3, F4, or F5 were considered anemic (positive test) and those classified as F1 and F2 were considered not anemic (negative test). Three gold standard Ht cutoff values were used to diagnose anemia, i.e., $\leq 22\%$, $\leq 18\%$, and $\leq 15\%$. In total, 1591 assessments were analyzed. The highest percentage of larvae in the fecal cultures was for *Haemonchus* spp., with an average of 78.5%. The sensitivity values found when F3 lambs were considered anemic were 13.9%, 30.8%, and 66.7%, respectively, for Ht cutoff values of $\leq 22\%$, $\leq 18\%$, and $\leq 15\%$. When F3 lambs were excluded, the maximum sensitivity was 14.9% and the specificity was 100% for all degrees of anemia using criterion 1. The FAMACHA© system had low sensitivity in growing lambs and should not be used alone for control of haemonchosis in young animals.

1. Introduction

Resistance to anthelmintic drugs is a worldwide problem in small ruminants, making it difficult to control gastrointestinal parasitosis and reducing the productive potential of flocks (Torres-Acosta et al., 2012). There are a number of reports of resistance to the major anthelmintics presently on the market (Waghorn et al., 2006; Papadopoulos, 2008; Scott et al., 2013; Cintra et al., 2016; Salgado and Santos, 2016).

Anthelmintic resistance progresses rapidly and indiscriminately, so alternative strategies that can be used in conjunction with anthelmintic activity are being sought to control gastrointestinal verminosis in small ruminants. One of these is targeted selective treatment (TST), whereby only animals identified to have a decline in productivity or clinical manifestations of verminosis are treated (Bath and Van Wyk, 2009).

The FAMACHA© system was developed to diagnose anemia in sheep and goats with haemonchosis and is used for TST in regions where the main parasite is *Haemonchus contortus*. Using this method, the color of the ocular mucosa is scored using the FAMACHA© chart, which contains a standardized set of five colors that represent different ranges of hematocrit (Ht) values as follows: F1, $> 28\%$; F2, 27%–23%; F3, 22%–18%; F4, 17%–13%; and F5, $< 12\%$ (Van Wyk and Bath, 2002).

Animals with a score of F4 or F5 are selected for anthelmintic treatment, and those with a score of F3 are investigated further to determine whether they need treatment or not (Van Wyk and Bath, 2002). The aim of this scoring method is to preserve the refugia population by decreasing the frequency of anthelmintic treatments, thereby delaying resistance to the active ingredients (Van Wyk, 2001; Kenyon et al., 2009; Bath, 2011; Hoste et al., 2011).

Since this method was implemented to control verminosis, several research groups have sought to determine if there are variations in the sensitivity and specificity of this strategy between breeds (Moors and Gaulty, 2009), production systems (Malan et al., 2001), animal categories (Mahieu et al., 2007), and species (Sotomaior et al., 2012). In adult animals, the FAMACHA© system is most frequently used to control verminosis in countries where there is a high prevalence of *H. contortus* (Vilela et al., 2008; Di Loria et al., 2009; Maia et al., 2014; Sotomaior and Cintra, 2016). However, there are few reports on the frequency of use or the efficiency of this system for selective anthelmintic treatment in young animals (Depner et al., 2007; Bentounsi et al., 2012; Fernandes et al., 2015). The objective of this study was to evaluate the sensitivity and specificity of the FAMACHA© system in growing lambs.

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2. Methodology

2.1. Animals

The study protocol was approved by the Ethics Committee on the Use of Animals at the Pontifical Catholic University of Paraná (number 0993/2015) and performed at two sheep farms in Paraná, Brazil. At the first farm, located in the Campos Gerais region, 80 purebred lambs (Texel and Ile de France) were evaluated from July 2015 to February 2016. At the second farm, located in the metropolitan region of Curitiba, 96 crossbred lambs (Texel and Ile de France) were evaluated from October 2015 to February 2016 and from June to September 2016.

2.2. Parasitologic and hematologic analyses

The lambs were followed from the age of 60 days until 210 days of life. Evaluations using the FAMACHA© system were performed at 15-day intervals by the same investigator along with parasitologic and hematologic analyses. The lambs were evaluated by comparing the color of the conjunctiva of the lower eyelid against the colors on the chart (F1–F5) using the method devised by Van Wyk and Bath (2002).

Feces were collected from each animal for measurement of the egg per gram of feces (EPG) count according to the method reported by Gordon and Whitlock (1939), which is sensitive for 50 EPG upwards. Larval cultures was performed monthly in fecal samples with an EPG > 500 using the method described by Roberts and O'Sullivan (1950).

Blood was collected from the external jugular vein in tubes containing ethylenediaminetetraacetic acid as the anticoagulant for the hematologic analysis. Hematocrit (Ht) was determined using the routine microhematocrit method reported by Jain (1986).

2.3. Assessment of sensitivity and specificity

There is no single Ht reference value available for diagnosis of anemia in sheep (Burke et al., 2007), so three gold standard Ht cutoff values for diagnosis of anemia were used to calculate the sensitivity and specificity of the FAMACHA© grading system. Ht values of $\leq 22\%$ and $\leq 18\%$ were chosen because they are the two extreme F3 values (Van Wyk and Bath, 2002) and an Ht value $\leq 15\%$ in sheep is considered to be life-threatening (Kaplan et al., 2004). Two criteria were considered in relation to the FAMACHA© system: criterion 1, lambs classified as F4 or F5 were considered anemic (positive test) and those classified as F1, F2, or F3 were considered not anemic (negative test); and criterion 2, lambs classified as F3, F4, or F5 were considered anemic (positive test) and those classified as F1 or F2 were deemed not to be anemic (negative test). A lamb was defined to be true-positive (TP) if it was classified as F3, F4, or F5 or as F4 or F5 and was found to have a Ht value of $\leq 22\%$,

$\leq 18\%$, or $\leq 15\%$. A false-positive (FP) lamb was defined as one that was classified as F3, F4, or F5 or as F4 or F5 but its Ht value did not indicate anemia. A lamb was deemed to be false-negative (FN) if it was scored as F1 or F2 or as F1, F2, or F3 but was found to be anemic on measurement of Ht. A true negative (TN) lamb was one that was scored as F1 and F2 or as F1, F2, or F3 and was not anemic on Ht testing.

The sensitivity (S) of the FAMACHA© system was calculated as the number of lambs that were considered anemic by this method as a proportion of the total number of lambs diagnosed as having anemia by gold standard Ht measurement, and was calculated using the following formula: $S = TP \times 100 / (TP + FN)$. The specificity (E) of the system was calculated as the proportion of lambs that were not classified as anemic by this method as a proportion of the total number of animals diagnosed as not being anemic on Ht measurement as follows: $E = TN \times 100 / (TN + FP)$.

The positive predictive value (PPV) indicated the probability that an animal would be anemic when the FAMACHA© score was positive for anemia ($PPV = TP \times 100 / (TP + FP)$) and the negative predictive value (NPV) indicated the probability that an animal was not anemic when the FAMACHA© score was negative for anemia ($NPV = TN \times 100 / (TN + FN)$) (Thrusfield, 2005).

The level of agreement between the Ht intervals and the FAMACHA© categories was calculated using the kappa (κ) statistic, where $\kappa = 0$ indicates agreement equivalent to that of chance, $\kappa = 1$ indicates complete agreement, $\kappa \geq 0.81$ indicates almost perfect agreement, $0.61 \geq \kappa \geq 0.80$ indicates substantial agreement, $0.41 \geq \kappa \geq 0.60$ indicates moderate agreement, $0.21 \geq \kappa \geq 0.40$ indicates reasonable agreement, and $\kappa \geq 0.2$ indicates weak agreement (Altman et al., 2000; Thrusfield, 2005).

To understand better the stage of life at which the S and E of the FAMACHA© system starts to increase or decrease, the S and E values were calculated a second time, only for criterion 2, and the lambs divided into two groups according to whether they were aged 60–89 days or 90–149 days.

A descriptive statistical analysis of the data was performed to compare the number of misdiagnoses made when F1, F2, F3, F4, and F5 scores were assigned and the Ht value was different from the standard value proposed by Van Wyk and Bath (2002). The EPG count and Ht value were evaluated in two ways, i.e., using the FAMACHA© score assigned by the evaluator and using the FAMACHA score and the Ht value according to Van Wyk and Bath (2002).

3. Results

Between 2015 and 2016, the larvae most commonly identified on fecal culture from both farms studied were *Haemonchus* spp; the mean value was 78.5%, but varied from 52.5% to 96% depending on the time of year (Fig. 1). *Trichostrongylus* spp. (15.6%) and *Oesophagostomum* spp. (8.5%) were also found.

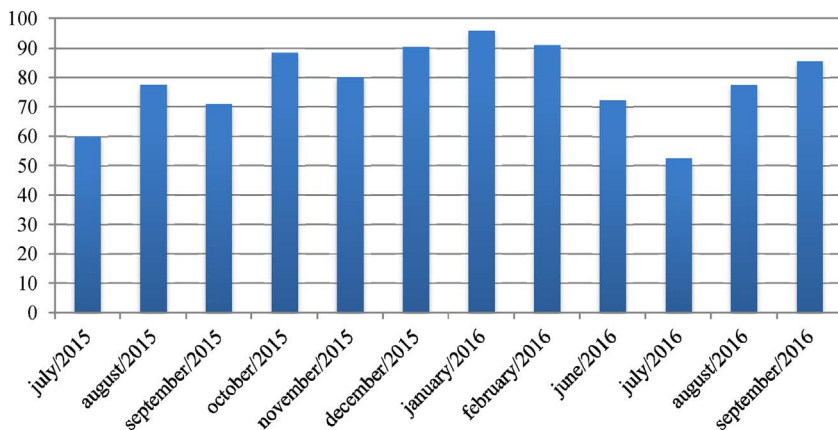


Fig. 1. Mean percentages of *Haemonchus* spp. larvae found in fecal cultures on two sheep farms in Paraná, Brazil, between 2015 and 2016.

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