Sensitivity and specificity of the FAMACHA© system in Suffolk sheep and crossbred Boer goats

Cristina Santos Sotomaior [a],*, Fernanda Rosalinski-Moraes[b], Alane Rayana Barbosa da Costa[a], Dhéri Maia[a], Alda Lúcia Gomes Monteiro[c], Jan A. van Wyk[d]

[a] *School of Agricultural Sciences and Veterinary Medicine – Pontifícia Universidade Católica do Paraná (PUCPR), São José dos Pinhais, Paraná 83010-500, Brazil*
[b] *Universidade Federal de Uberlândia (UFU), Faculdade de Medicina Veterinária – FAMEV, Uberlândia, Minas Gerais 38405-315, Brazil*
[c] *Universidade Federal do Paraná (UFPR), Setor de Ciências Agrárias, Departamento de Zootecnia, Curitiba, Paraná 80035-050, Brazil*
[d] *Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria, P/B X04, 0110 Onderstepoort, South Africa*

* Corresponding author at: Programa de Pós-Graduaç̃ão em Ciência Animal, Escola de Ciências Agrárias e Medicina Veterinária, Pontifícia Universidade Católica do Paraná – PUCPR, Rodovia BR 376 – Km 14, São José dos Pinhais, Paraná 83010-500, Brazil. Tel.: +55 41 3299 4437; fax: +55 41 3299 4363.

E-mail addresses: cristina.sotomaior@pucpr.br (C.S. Sotomaior), fermoraes@famev.ufu.br (F. Rosalinski-Moraes), alane_rayana@yahoo.com.br (A.R.B. da Costa), dherimaia@hotmail.com (D. Maia), aldaufpr@gmail.com (A.L.G. Monteiro), jan.vanwyk@up.ac.za (J.A. van Wyk).

Abstract

Sheep and goats are the species of farm animal with the highest growth rate in Paraná State. The main problems facing Paraná State flocks are gastrointestinal parasites and anthelmintic resistance. One of the newest resources used to slow down the development of anthelmintic resistance is the FAMACHA© system, a selective method useful for controlling gastrointestinal verminosis in small ruminants. The purpose of the present research was to evaluate the sensitivity and specificity of the FAMACHA© system in sheep and goats and to compare the results for both species. The conjunctivae of 83 Suffolk ewes and 60 adult cross-bred Boer does were evaluated by the same trained person using the FAMACHA© system. The packed cell value (PCV) served as the gold standard for clinical FAMACHA© evaluation. To calculate the sensitivity and specificity of the FAMACHA© system, different criteria were adopted in turn: animals classified as FAMACHA© (F©) 4 and 5, or 3, 4 and 5, were consid-ered to be anemic (positive test), and animals classified as F©1, 2 and 3, or 1 and 2 were considered to be non-anemic (negative test). Three standard values of PCV, namely $\leq 19\%$, $\leq 18\%$ or $\leq 15\%$, were used to confirm anemia. At all cut-off levels, the sensitivity increased if F©3 animals were included as being anemic. However, changes in levels of sensitivity were associated with reciprocal changes in specificity. The sensitivity was higher for sheep than for goats, excepting when the criteria included PCV ≤ 18 and F©3, F©4 and F©5 were considered positive. In contrast, the specificity was always lower in sheep for any criteria adopted. Other than in goats, using the ≤ 15 cut-off level for sheep, it is possible to opt not to drench the animals that were shown to be F©3 because the sensitivity is still high, indicating that few animals that should have been drenched were overlooked. In goats, in contrast, the low sensitivity at all cut-off levels made it too risky to leave F©3 animals undrenched. Even though the number of correct treatments for goats was always higher than that for sheep, the opposite was true for the kappa index for all the criteria tested. Therefore, the FAMACHA© system is suitable for the identification of anemic animals of both species. It is necessary that all small ruminants classified as FAMACHA© level 3 are also treated to increase the sensitivity of the method.

Keywords:
Targeted selective treatment FAMACHA©
Haemonchus
Worm control
Sheep
Goats

1. Introduction

Sheep and goats are the species of farm animals with the largest growth rate in Paraná State between 2004 and 2010, with increases of 26% from 488,000 to 614,000 in sheep and 90% from 96,000 to 182,000 in goats (IBGE, 2010). Most sheep and goats are raised on small and medium-sized farms in intensive or semi-intensive systems characterized by high stocking rates of 20–50 sheep/goat per hectare, and slaughter lamb and kid production is the primary economic endeavor. However, this is increasingly coming under threat from developing resistance of *Haemonchus contortus* to anthelmintics, since this parasite species constitutes one of the main problems of sheep and goat farming in Parana State (Depner et al., 2007).

The evolution of drug-resistant nematode populations throughout the world is well known (Kaplan, 2004; Papadopoulos, 2008). In Paraná State, the indiscriminate use of anthelmintics, including blanket treatment of all animals at fixed intervals as short as monthly, bimonthly or even weekly, and treating the entire group when one or more animals demonstrate clinical helminthosis has resulted in high levels of parasitic resistance to all the anthelmintic activity groups available in Brazil for use in sheep flocks (Thomaz-Soccol et al., 2004).

When an entire flock is treated, there can be great selection pressure for resistance in nematode populations, depending on the levels of refugia at the time. It is now recognized that the proportion of a given helminth population under drug selection is possibly the single most important factor that influences the rate at which resistance will develop. Therefore, nematode control programs should be designed to maintain the maximum amount of refugia (the portion of the population that is not exposed to the drug) that is commensurate with sustain-able parasite management and animal production (Van Wyk, 2001).

Researchers worldwide have sought to develop practical methods of integrated parasite management (IPM) for reducing anthelmintic drug usage (Bath, 2011; Hoste et al., 2011). One such aid is the FAMACHA© (F©) system, a method of clinical evaluation of anemia, used primarily for selective anthelmintic treatment of only those individual animals which cannot manage unaided under field conditions of severe *H. contortus* challenge (Bath et al., 2001; Van Wyk and Bath, 2002). Through clinical identification and selective treatment of overly susceptible hosts, while leaving the resistant and resilient ones (i.e. those which are, respectively, able either to eliminate parasites or to withstand their effect), use of anthelmintic drugs can considerably be reduced (Malan et al., 2001; Van Wyk and Bath, 2002; Mahieu et al., 2007; Molento et al., 2009).

Since its induction the FAMACHA© system has been studied in a variety of different countries and production systems to optimize its use (Malan et al., 2001; Vatta et al., 2001; Kaplan et al., 2004; Ejlertsen et al., 2006; Di Loria et al., 2009; Molento et al., 2009; Riley and Van Wyk, 2009; Scheuerle et al., 2010). Possible variations among breeds (Moors and Gauly, 2009), animal categories and ages (Mahieu et al., 2007), evaluators (Burke et al., 2007), management systems (Reynecke et al., 2011b), environments

and facilities must be investigated to determine the real limitations of this method.

In the light of the suggestion of Vatta et al. (2001) that the FAMACHA© technique could be less accurate in goats than in sheep, the present research was conducted to compare the sensitivity and specificity of the FAMACHA© system in these two species.

2. Materials and methods

The research was performed at LAPOC (Laboratório de Produc̨ão e Pesquisa em Ovinos e Caprinos – Laboratory for the Production and Research of Sheep and Goats), located in the Cangüiri Center of Experimental Stations, Federal Uni-versity of Paraná, and in the Hospital Unit for Farm Animals at the Pontifical Catholic University of Paraná, from October 5, 2009 to November 20, 2009.

Eighty-three 2–8 year-old Suffolk ewes and 60 2–7 year-old crossbred Boer does were evaluated using the FAMACHA© system. While the ewes were toward the end of their period of lactation and grazed low quality Tifton pasture, only 20% of the does were similarly lactating, also on Tifton pasture, but the latter was of better quality and availability than that of the sheep. For both host species this evaluation was always performed by the same previously trained person according to Van Wyk and Bath (2002) by comparing the color of the conjunctiva to the appropriate FAMACHA© chart of Bath et al. (2001). At each evaluation occasion blood was collected for determining the packed cell volume (PCV) of every animal.

The FAMACHA© categories and their respective PCV values were analyzed according to Bath et al. (2001). For the calculation of the sensitivity and specificity of the FAMACHA© system, two different criteria were adopted:(I) animals classified as FAMACHA© 4 and 5 were con-sidered to be anemic (positive test) and FAMACHA© 1, 2 and 3 non-anemic (negative test); (II) animals classified as FAMACHA© 3, 4 and 5 were considered to be anemic (positive-test) and FAMACHA© 1 and 2 non-anemic (negative test). For the PCV, the standard test used to confirm anemia, three different values were used ($\leq$19%, $\leq$18% or $\leq$15%), as no precise value for PCV has been clearly established at which anemia crosses the threshold of clinical importance (Kaplan et al., 2004; Burke et al., 2007). A true positive (TP) result was defined as animals that were anemic (PCV $\leq$15, $\leq$18 or $\leq$19%) with pale eye scores (4, 5 or 3, 4, 5). A false positive (FP) result was defined as animals that were not anemic (PCV >15, >18 or >19%) with pale eye scores. A false negative (FN) result was defined as animals that were anemic with red or pink eye scores (1, 2 or 1, 2, 3). A true negative (TN) result was defined as animals that were not anemic with pink or red eye scores.

Sensitivity, specificity, the predictive value of a negative and the predictive value of a positive were calculated according to Vatta et al. (2001) and Thrusfield (2005). Sensi-tivity (Se) is the proportion of infected or diseased individu-als with a positive test or true positive (TP), or in the case of the FAMACHA© clinical assay, the proportion of anemic ani-mals correctly identified as anemic, above all real anemic animals [Se = TP $\times$ 100/(TP + FN)]. Specificity (Sp) is defined as the proportion of disease-free individuals that test

Table 1
The distribution of sheep and goats as recorded by FAMACHA© category.

FAMACHA©	Sheep		Goats	
	n	%	n	%
1	16	19.2	22	36.7
2	29	35.0	25	41.6
3	26	31.3	12	20.0
4	11	13.3	01	1.7
5	01	1.2	00	0.0
Total	83	100.0	60	100.0

negative or true negative (TN), i.e. the proportion of non-anemic animals that are correctly categorized as such, above all real non-anemic sheep or goats [$Sp = TN \times 100/(TN + FP)$]. In the case of the FAMACHA© system, the predictive value of a negative (PVN) is the probability that an animal is not anemic when the test result is negative for anemia, and vice versa for the predictive value of a positive (PVP) [$PVN = TN \times 100/(TN + FN)$; $PVP = TP \times 100/(TP + FP)$].

To evaluate the association between FAMACHA© scores and PCV, the kappa (κ) value was calculated, as described in Thrusfield (2005). The κ values were ranked in the following manner: >0.80, very good agreement; 0.61–0.80, good agreement; 0.41–0.60, moderate agreement; 0.21–0.40, fair agreement; and <0.2, poor agreement (Altman et al., 2000).

3. Results

The distribution of the animals in the five FAMACHA© categories was different in the two species (Table 1). For sheep, less than 20% of the animals were classified as FAMACHA© 1 (F©1), and 45.8% were recorded as F©3, F©4 and F©5. For goats, most of the animals were classified as F©1 and F©2 (78.3%).

The percentage of correct treatments was always higher for goats (Table 2), although the kappa index was lower than sheep for all the criteria tested (Table 3). The kappa index ranged between 0.22 and 0.74, indicating from fair to good agreement. The prevalence (estimated by the PCV values) and the positive and negative predictive values are listed in Table 3.

The sensitivity ranged from 16.7% to 100% and the speci-ficity from 62.5% to 100%, depending on the species and the criteria used (Table 4). The results showed that includ-ing F©3 as anemic increased the sensitivity and reduced the specificity for all PCV cut-off values for both sheep and goats.

4. Discussion

There have been several studies involving the application of the FAMACHA© system separately to either sheep (Burke and Miller, 2008; Molento et al., 2009; Reynecke et al., 2011a,b), or goats (Vatta et al., 2001; Mahieu et al., 2007; Scheuerle et al., 2010), but relatively few including both (Kaplan et al., 2004; Burke et al., 2007). Usually, different percentages of sensitivity and specificity are found, depending on the criteria employed, the management

system, the evaluators' experience and the prevalence of anemic individuals.

The present study used different criteria for calculating the sensitivity and specificity of the FAMACHA© system, depending on whether or not F©3 animals were included in the positive test group (anemic), as suggested by Vatta et al. (2001). Because no precise value for the PCV at which anemia crosses a threshold of clinical importance has been clearly established (Burke et al., 2007), different criteria for the PCV cut-off level for anemia confirmation were used. A cut-off level of 19% for anemia was selected because, in an epidemiological study, Jain (1986) listed normal PCVs for goats as 19–34%. In addition, Vatta et al. (2001) suggest that a PCV level of 18% must be considered to be the cut-off level because it is the limiting value between categories 3 and 4 of the FAMACHA© system. On the other hand, studies by Kaplan et al. (2004) show that an animal with a PCV as low as 15% is not necessarily clinically ill and at risk of death. Such variation in cut-off levels causes great variations in the results, hence the three cut-off values of $\leq$19%, $\leq$18% and $\leq$15% were selected for defining anemia in this study.

While there were important differences in the management of the sheep and goats in terms of their reproductive classes and nutrition and the average levels of anemia that developed, this study had the advantage that clinical evaluations of both host species were done by the same person. In sheep, only 19.3% of animals were classified as F©1, and almost 15% were classified as F©4 and F©5, in comparison with 78.3% of the goats classified as F©1 and F©2, only 1.7% as F©4, and none as F©5 (Table 1). It seems likely that the differences in reproductive stage and the better quality of pasture of the goats than that of the sheep in the present study could explain the differences in the distribution of animals in the FAMACHA© categories (Malan et al., 2001) as well as in the higher levels of anemia in sheep than in goats with respective means of 45.8% and 21.7% in cate-gories 3–5. It is well known that nutrition is a key factor in immunity and resilience to gastrointestinal nematodes (Kyriazakis and Houdjk, 2005; Knox et al., 2006).

Excepting when the parameter of a PCV $\leq$ 18 was used and F©3, F©4 and F©5 were considered to be positive tests, the FAMACHA© sensitivity was always higher in sheep than in goats. Specificity, on the other hand, was always lower in sheep, regardless of the criteria adopted. For all the criteria tested, the kappa index was lower in goats, although the number of correct treatments for this species was always higher than for sheep, possibly due to the lower prevalence of anemic goats.

Regarding the sensitivity and specificity tests, the differences between the results were most evident when F©3 animals were considered to be anemic. In agreement with the results of Kaplan et al. (2004) and Burke et al. (2007), the sensitivity for both sheep and goats increased at all cut-off levels when F©3 animals were included as being anemic (Table 4), although with a corresponding decrease in specificity. In contrast, sensitivity increased for both species when the criterion of classifying anemia according to the PCV was less strict. This means that sensitivity may reach 100% and the number of false negatives could drop to zero if a PCV of 15% is considered as the cut-off level and cat-egory 3 of the FAMACHA© system is considered positive,

Table 2

The number of false-negative, false-positive, true-negative, true-positive and correct treatment results of the FAMACHA© system, according to different evaluation criteria, for sheep and goats.

	False-negative	False-positive	True-positive	True-negative	Correct treatment[a]
Sheep					
FAMACHA© values 3, 4, 5 considered as positive tests					
PCV[a] ≤ 15	0 (0%)	27 (32.5%)	11 (13.3%)	45 (54.2%)	56 (67.5%)
PCV ≤ 18	1 (1.2%)	22 (26.5%)	16 (19.3%)	44 (53.0%)	60 (72.3%)
PCV ≤ 19	2 (2.4%)	20 (24.1%)	18 (21.7%)	43 (51.8%)	61 (73.5%)
FAMACHA© values 4, 5 considered as positive tests					
PCV ≤ 15	2 (2.4%)	3 (3.6%)	9 (10.9%)	69 (83.1%)	78 (94.0%)
PCV ≤ 18	7 (8.4%)	2 (2.4%)	10 (12.1%)	64 (77.1%)	74 (89.2%)
PCV ≤ 19	9 (10.8%)	1 (1.2%)	11 (13.3%)	62 (74.7%)	73 (88.0%)
Goats					
FAMACHA© values 3, 4, 5 considered as positive tests					
PCV[a] ≤ 15	0 (0%)	11 (18.3%)	2 (3.3%)	47 (78.4%)	49 (81.7%)
PCV ≤ 18	0 (0%)	10 (16.7%)	3 (5.0%)	47 (78.3%)	50 (83.3%)
PCV ≤ 19	2 (3.3%)	9 (15.0%)	4 (6.7%)	45 (75.0%)	49 (81.7%)
FAMACHA© values 4, 5 considered as positive tests					
PCV ≤ 15	1 (1.7%)	0 (0%)	1 (1.7%)	58 (96.6%)	59 (98.3%)
PCV ≤ 18	2 (3.3%)	0 (0%)	1 (1.7%)	57 (95.0%)	58 (96.7%)
PCV ≤ 19	5 (8.3%)	0 (0%)	1 (1.7%)	54 (90.0%)	55 (91.7%)

[a]PCV, packed cell volume.
[b]Correct treatment – true-positive + true-negative.

Table 3

The prevalence[a] of anemic animals, the positive and negative predictive value of the FAMACHA© system, and the kappa (κ) value between the packed cell volume (PCV) and FAMACHA© system for sheep and goats.

	Prevalence sheep (%)	PPV[b] sheep (%)	NPV[c] sheep (%)	Kappa sheep	Prevalence goats (%)	PPV[b] goats (%)	NPV[c] goats (%)	Kappa goats
FAMACHA© values 3, 4, 5 considered as positive tests								
PCV[d] ≤ 15	13.3	29.0	100.0	0.306	3.3	15.4	100.0	0.222
PCV ≤ 18	20.5	42.1	97.8	0.417	5.0	23.1	100.0	0.320
PCV ≤ 19	24.1	47.4	95.6	0.446	10.0	30.8	95.7	0.329
FAMACHA© values 4, 5 considered as positive tests								
PCV ≤ 15	13.3	75.0	97.2	0.748	3.3	100.0	98.4	0.659
PCV ≤ 18	20.5	83.3	90.1	0.626	5.0	100.0	96.6	0.487
PCV ≤ 19	24.1	91.7	87.3	0.619	10.0	100.0	91.5	0.265

[a] Estimated by PCV value.
[b] PPV, positive predictive value of FAMACHA©.
[c] NPV, negative predictive value of FAMACHA©.
[d] PCV, packed cell volume.

as also found for both host species by Kaplan et al. (2004). It also ratifies the decision of Van Wyk and Bath (2002) to recommend routine treatment of every animal judged to be in FAMACHA© categories 3–5.

Higher sensitivity values are more important than higher specificity values in the FAMACHA© system. Not treating the animals that are truly in need of treatment (false negatives) exposes them to the risk of death, while treating animals that do not need treatment (false positives) does not cause serious problems (Vatta et al., 2001; Kaplan et al., 2004). In this respect, the false negative results of the two host species were generally very similar, excepting when FAMACHA© values of only 4 and 5 were considered as positive for anemia. However, it seems likely that this is one aspect where the large difference in the average levels of anemia could have played a crucial role.

When the F©3 category was not included as ane-mic, κ values were higher, indicating moderate to good agreement (Table 3). This change in the κ value was seen primarily in sheep because there was an important

Table 4

The sensitivity and specificity of the FAMACHA© system, according to different evaluation criteria, for sheep and goats.

	Sensitivity sheep	Specificity sheep	Sensitivity goats	Specificity goats
FAMACHA© values 3, 4, 5 considered as positive tests				
PCV ≤ 15	100.0	62.5	100.0	81.0
PCV ≤ 18	94.1	66.7	100.0	82.5
PCV ≤ 19	90.0	68.3	66.7	83.3
FAMACHA© values 4, 5 considered as positive tests				
PCV ≤ 15	81.8	95.8	50.0	100.0
PCV ≤ 18	58.8	97.0	33.3	100.0

decrease in the percentage of false positives, and thus a higher proportion of correct treatments (Table 2). However, there was an accompanying reciprocal increase in the false negatives, which is very undesirable. In contrast, inclusion of the F$^{©}$3 category as anemic is inclined to lead to an increase in the proportion of unnecessary drenches, although as described by Reynecke et al. (2011a) this is less harmful as regards selection for anthelmintic resistance than traditional whole-mob use of anthelmintics and does not negatively affect the method; even with an increase in the use of anthelmintics, there is still a considerable reduction in drug use, thus making it an important tool for maintaining a population of *H. contortus* in refugia (Kaplan et al., 2004; Mahieu et al., 2007; Burke et al., 2007).

Many other workers also recommend inclusion of F$^{©}$3 animals as needing treatment (Kaplan et al., 2004; Mahieu et al., 2007), or even F$^{©}$2 and eventually all if average levels of anemia in a given flock or herd continue to rise despite treatment of all animals in F$^{©}$ categories 3–5 (Van Wyk and Bath, 2002; Reynecke et al., 2011a,b), and/or indicate that evaluations of the flock should take place more often to increase the sensitivity of the method (Burke et al., 2007; Mahieu et al., 2007; Reynecke et al., 2011a). One of the main advantages of the FAMACHA$^{©}$ system as a diagnostic test is that it can be adjusted for arbitrary definitions for the determination of sensitivity and specificity, as it has five categories that allow different views of the infection status of a flock and thus allows upward or downward adjustment of categories to treat or leave untreated (Reynecke et al., 2011a).

5. Conclusions

Despite the fact that the goats in the study were not exposed to similar levels of haemonchosis to serve as a good comparison with the sheep, more of which developed relatively severe anemia, we concur with the conclusions of Mahieu et al. (2007) concerning goats, and that the FAMACHA$^{©}$ method can be used as a safe and reliable approach to reducing pressure in sheep and goats on the selection for anthelmintics in relation to routine non-selective blanket treatment for worm control.

The present results strongly support the stipulation by Van Wyk and Bath (2002) and more recent workers that it is necessary routinely to deworm all animals scored as FAMACHA$^{©}$ level 3 together with those in categories 4 and 5 for optimizing the sensitivity without having an important effect on the rate of selection for anthelmintic resistance despite treatment of larger proportions of animals than when those in FAMACHA$^{©}$ category 3 animals are excluded.

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