Application of ROC curve analysis to FAMACHA® evaluation of
haemonchosis on two sheep farms in South Africa

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Abstract

Test sensitivity and specificity for the FAMACHA© clinical test for anaemia due to haemonchosis have previously been shown to be adequate in differentiating between heavily/less heavily infected sheep, but these properties give no objective guidance for setting the optimum threshold at which anthelmintic treatment should occur. The aim of this work was to use Receiver Operating Characteristic curves (ROC) to evaluate the diagnostic accuracy of FAMACHA© testing by estimating the area under the ROC curve, and to use Two-graph ROC curves to decrease subjectivity in selecting treatment thresholds on two farms with contrasting management. Test diagnostic accuracy, and thus discriminating power as determined by the area under the ROC curves, ranged from “moderate to good” on the first farm, and from “moderate to high” on the second farm for haematocrit (the Gold Standard for the test) cut-offs of $\leq 22\%$ and $\leq 19\%$ on both farms respectively. Accuracy of classification between haematocrit cut-offs was not significantly different within farms, but did differ significantly between farms, with test accuracy being highest on the second farm at both haematocrit cut-offs ($p< 0.05$). The results also showed the suitability of the two-graph ROC curve approach for discriminating not only between different levels of accuracy of evaluators, but also to give an indication of the so-called ROC cut point (i.e. the desired threshold level) at which animals should be treated for a given level of risk of loss. The approach appears to have the potential not only to validate the diagnostic accuracy of the test across the complete testing range (i.e. all FAMACHA© categories from 1 – 5), but also to compensate for such inaccuracy by allowing objective adjustment of the threshold treatment level according to the output of the two-graph ROC method.
1. Introduction

Targeted selective treatment (TST), based on the concept of “refugia” (Martin, 1989; Van Wyk, 2001; Kenyon et al., 2009) for more sustainable worm management was previously not practicable until it was given substance by the FAMACHA© system of clinical evaluation of the anaemia of haemonchosis (Bath et al., 2001; Van Wyk, 2001; Vatta et al., 2001; Kaplan et al., 2004; Mahieu et al., 2007; Molento et al., 2004; Sotomaior et al., 2004). The FAMACHA© system is based on leaving untreated all animals that are not clinically affected by haemonchosis at any given time. It thus fits the present paradigm of refugia, by allowing the relatively large numbers of worms in such untreated animals to reproduce unhindered. However, the system has the disadvantage that decisions on the treatment threshold of animals in a given flock are relatively subjective. An important factor in the application of the FAMACHA© test is that production losses inevitably occur before positive test results are obtained (FAMACHA© being a so-called “lagging” indicator); this is now an accepted part of sustainable parasite management in that a proportion of production is lost to sustainable parasite management, even though present indications are that the losses may be smaller than initially expected (Mahieu et al., 2007; Van Wyk, 2008; Molento et al., 2009). Therefore, given that selective treatment involves treating only individuals deemed not to be coping with
infection, it is important that (i) test diagnostic accuracy is maximised and (ii) treatment thresholds are accurately selected according to the prevailing epidemiological situation and accuracy of test implementation.

Sensitivity (Se) and specificity (Sp) analysis has proven to be useful for reducing the subjectivity of FAMACHA© application, but falls short of providing threshold values for discriminating between categories of animals which either require treatment or can be left untreated. In this paper ROC analysis (Zweig & Campbell, 1993) was used in combination with the Likelihood Ratio of a Positive Test Result (LR+) for setting cut points for FAMACHA© treatment thresholds for given sets of conditions. The LR+ is a combined measure of Se and Sp (Greiner and Gardner 2000), and can be used to test the strength of the relationship between the test result and the probability of disease (Smith, 1995). The likelihood ratio for a positive test result (LR+) describes how much the odds of the disease increase when a test is positive.

2. Materials and methods

In this work, the term “cut-off” is used to classify the disease status of an animal according to a preselected haematocrit value into diseased/non-diseased. The “ROC cut point” refers to dichotomised FAMACHA© test results, i.e. to designate the FAMACHA© categories as rating points (Hanley and McNeil, 1982), that indicate the positive or negative test status of an individual. The cut points were used to calculate the area under the ROC curve index values non-parametrically in STATA (Version 8.0; STATA Corporation) for selected haematocrit cut-off values. For example, if all individuals in
FAMACHA© categories 2–5 are considered to be test positive, then the ROC cut point is 2, and if all individuals in FAMACHA© categories 3–5 are considered test positive, the ROC cut point is 3, etc. A further refinement of ROC analysis, the two-graph ROC curve method (Greiner et al., 1995; 2000; Beck et al., 2005), was used to optimise the selection of FAMACHA© ROC cut points for anthelmintic treatment by plotting each Se and Sp curve individually, as a function of the FAMACHA© ROC cut points.

2.1. Origin of data and test procedures

The data analysed consisted of anaemia status as evaluated by FAMACHA© scores and haematocrit values, collected from naturally infected sheep involved in two commercial farm trials in the summer rainfall region of South Africa. In this region the Haemonchus season corresponds with the summer rainfall period, from September-November until April-May the following year.
Faecal worm egg counts (FECs) were done at irregular intervals, using a sucrose flotation modification of the McMaster method (Reinecke 1973), at a sensitivity of 100 eggs per gram of faeces. Distance from the laboratory precluded regular worm egg counts and faecal cultures. However, the tests that were done, together with post mortem examinations done during intermittent visits by the research team over the trial periods, (including haematocrit determinations) indicated a preponderance of \textit{H. contortus} infections annually during the period from December to the end of February on both farms, with \textit{Trichostrongylus colubriformis} and \textit{Teladorsagia circumcincta} being relatively prevalent thereafter on Farm 1 (below), in late autumn and winter. Furthermore, on both farms the preponderance of \textit{H. contortus} infection was confirmed by “diagnostic treatment” (Gordon, 1981), in that the anaemia of animals which were treated for haemonchosis, was promptly cured and highly significant levels of correlation between haematocrit and FAMACHA© values in the sheep were observed (Riley and Van Wyk, 2009).

\textbf{Farm 1}: The FAMACHA© system was tested on this farm over a period of five years in stud Merino sheep under routine farming conditions (Riley and Van Wyk, 2009; Reynecke et al., 2010 \textit{in press}). Approximately 130–260 young replacement sheep of each class (sex) out of a total of 1500 sheep on this farm were FAMACHA©-evaluated 7-11 times per worm season. After initial deworming of all animals in the trial, only those clinically judged to be in FAMACHA© categories 3–5 were dewormed routinely. When
“severe worm challenge” was again evident, all sheep were again dewormed\textsuperscript{1}, and the process repeated. Severe worm challenge was defined as a quarter of all animals being scored into the high-risk, moderately to highly anaemic FAMACHA© categories 3, 4 and 5; this occurred usually in January or February of each year. The intervals between FAMACHA© evaluation mostly varied from 3-4, or rarely 5 weeks, during the various trials. On several occasions during the course of the trials, blood samples were collected from each animal for haematocrit determination (Table 1). FAMACHA© evaluation of the sheep was done throughout by the same individual, who had been given basic training in the use of the system (Bath et al., 2001; Van Wyk et al., 2001).

Farm 2: This trial was conducted on a Merino sheep farm to which rams from a variety of farms were congregated for comparing their production under identical conditions on natural pasture. FAMACHA© evaluation was done fortnightly on the ram intakes over two consecutive years (i.e. two \textit{Haemonchus} seasons). Haematocrit determinations were done on all rams, both at the beginning and at the height of each \textit{Haemonchus} season in January of each year (Table 1). Every ram judged during fortnightly evaluations over each \textit{Haemonchus} season from October to the following April by the farmer to be in FAMACHA© category 4 or 5 was bled for haematocrit determination, and only rams with haematocrit values of 15 % or lower were dewormed. Sheep on this second farm were also scored by only one investigator, with the exception of the first three evaluations in the first season, when FAMACHA© classifications were the collective observations by

\textsuperscript{1} This was done to end each period of FAMACHA© evaluation, so that the animals could be prepared for evaluation of their breeding values for production, including wool characteristics (Riley and Van Wyk, 2009)
him and 1–3 other persons.

Table 1 Criteria for anthelmintic treatment, number of FAMACHA© evaluations where haematocrits were determined and total number of haematocrit determinations for the two farms in the analysis.

<table>
<thead>
<tr>
<th></th>
<th>Criteria for treatment of individual animals</th>
<th>Number of FAMACHA© evaluations which included haematocrit determination</th>
<th>Total number of haematocrit determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm 1</td>
<td>Only treated if scored into FAMACHA© 3, 4 or 5</td>
<td>2000: 263</td>
<td>675</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2001: 160</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2002: 127</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2003: 125</td>
<td></td>
</tr>
<tr>
<td>Farm 2</td>
<td>Only treated if in scored into FAMACHA© 4 or 5 and haematocrit ≤ 15%</td>
<td>2000: 132</td>
<td>806</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2001: 319</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>2002: 355</td>
<td></td>
</tr>
</tbody>
</table>
2.2. Receiver Operating Characteristic (ROC) curve analysis

The area under the ROC curve was non-parametrically calculated for two haematocrit cut-off values (≤22 % and ≤19 %) and for FAMACHA© data from each farm, using STATA Version 8 (STATA Corporation, College Station, Texas). A distinction is made between non-informative tests (area under curve = 0.5), tests of low accuracy (0.5<area under curve<0.7), moderately accurate tests (0.7<area under curve<0.9) and highly accurate tests (0.9<area under the curve<1) (Swets, 1988). The area under the ROC curve thus measures the ability of the test to discriminate - i.e., the accuracy of the test to correctly classify those either with or without the disease concerned.

STATA Version 8 was also used to calculate Se, Sp and LR+ using the rating method (Hanley and McNeil, 1982). The haematocrit cut-off value of ≤22 % was chosen because it is the upper haematocrit limit for FAMACHA© category 3, which is recommended as a treatment threshold during the application of the FAMACHA© system (Van Wyk and Bath 2002). However, a cut-off value ≤19 % was included in addition to provide an additional view of the data, since a small proportion of animals with a haematocrit of ≤19 % could develop terminal anaemia within seven days if not detected and treated (Malan et al., 2001), as well as to determine if there would be significant differences in accuracy of FAMACHA© testing between the selected haematocrit cut-offs on each farm.

The haematocrit ranges for each of the five FAMACHA© categories were used as the “Gold Standard” to validate the respective FAMACHA© categories for two levels of infection on the two farms, namely either FAMACHA© categories 2–5, or FAMACHA©
categories 3–5 regarded as test positive. The points required to produce the ROC curve were obtained by successively considering increasingly broader categories of abnormal test results, for example by considering FAMACHA© category 5 alone as abnormal, then FAMACHA© category 5 plus 4, then FAMACHA© category 5 plus 4 plus 3, etc. Two-graph ROC analysis (a plot of both Se and Sp against FAMACHA© test cut-point) was then used to determine the FAMACHA© ROC cut points which would yield a Se of ≥80 %, combined with an acceptable Sp (i.e. leaving untreated, at least 50 % of animals which do not require treatment), while keeping the potential penalty of non-treatment of a severely anaemic animal in mind. Hence the ROC cut points were selected to ensure that a minimum of 80 % of animals defined as diseased according to the results of FAMACHA© evaluation, would be detected and treated in both trials. Sp of FAMACHA© evaluation was deemed to be acceptable if at least 50 % of animals not requiring treatment were detected by the evaluation, and were therefore correctly left untreated. The LR+ was calculated to express the odds that an animal that tested positive at a given threshold, was truly diseased or not.

3. Results

For Farm 1, the area under the ROC curve for haematocrit cut-offs of ≤22 % and ≤19 % were respectively, 0.79 and 0.83, indicating moderate accuracy (see above) in discriminating between anaemic and non-anaemic individuals (Fig. 1a). In contrast, for Farm 2, the discriminating power was moderate to high, with corresponding values of 0.86 and 0.90, (Fig. 1b). No significant differences in accuracy of diagnosis between the
two selected haematocrit cut-offs were detected within farms, as indicated by overlapping 95% confidence limits (Fig. 1). However, differences in diagnostic accuracy were significant between farms (p < 0.05) for the same haematocrit cut-offs, with the accuracy of FAMACHA© classification being significantly higher on Farm 2 at both haematocrit cut-offs (Fig. 1).

The results of the two-graph ROC analysis are given in Fig. 2. Se was higher on Farm 2 for any given ROC cut point and haematocrit cut-off (Fig. 2a-2d), while Sp did not vary greatly between farms. For Farm 1, test accuracy was maximised at a ROC test cut point of 2 and a haematocrit Se/Sp cut-off of ≤22% (i.e. the ROC cut point with the highest average value for Se + Sp ) (Fig. 2a), while for Farm 2 Se and Sp were optimised at a ROC cut point of FAMACHA© category 3 (Fig. 2d). LR+ ratios for all ROC cut points are given in Table 2, which shows that the highest LR+ value obtained for Farm 1 was 10.47 for a test ROC cut point of 3 at a haematocrit cut-off of ≤22%. For Farm 2, on the other hand, the highest LR+ value was 49.01 for a FAMACHA© test ROC cut point of 4 and a haematocrit cut-off of ≤22%.
b.

FIG. 1. Receiver Operating Characteristic (ROC) curves. (a) Farm 1: AUC is 0.79 (0.75 – 0.82*) at a haematocrit cut-off of ≤22 % and 0.83 (0.80 – 0.86*) at a cut-off of ≤19 %. (b) Farm 2: AUC is 0.86 (0.84 – 0.88*) for a cut-off of ≤22 % and 0.90 (0.87 – 0.92*) for a cut-off of ≤19 %.

* lower and upper 95 % confidence limit; Se – sensitivity.
FIG. 2. Two-graph Receiver Operating Characteristic curve (ROC) plots for various haematocrit cut-off values. (a) and (b) Farm 1, haematocrit cut-offs of \( \leq 22\% \) and \( \leq 19\% \), respectively; (c) and (d) Farm 2*, haematocrit cut-offs of \( \leq 22\% \) and \( \leq 19\% \), respectively

*FAMACHA© Category 5 not represented; Se – sensitivity; Sp - specificity
4. Discussion

4.1 ROC analysis as a tool in FAMACHA© evaluation of the anaemia of haemonchosis

The advantage of ROC over use of only a measure of test Se and Sp, is that a definite ROC cut point is obtained, indicating for a given set of circumstances and evaluators, which categories of animals should be treated and which could safely be left untreated. Setting treatment thresholds according to quantitative criteria, such as the recommendation that test Se should be at least 0.8 under any criteria, is in contrast to the present subjective approach to deciding on the categories of animals to treat or leave untreated. Risk of production losses could largely be reduced following this approach, for instance by allowing for an evaluator who consistently under- or overestimates the anaemia status of individual animals. All ROC area under curve values obtained in this study were much larger than 0.5 (i.e. larger than the maximum accepted value for non-discrimination), indicating that the discriminating power for the FAMACHA© test was acceptable for both of the haematocrit cut-off values.

On Farm 1 the area under the curve for both haematocrit cut-off values was slightly lower than for the corresponding cut-off values on Farm 2, thus accuracy of FAMACHA© classification was lower on Farm 1 than on Farm 2 (Figs. 1 a and b). The higher overall test accuracy on Farm 2 was expected, since FAMACHA© evaluation was not only done more frequently on Farm 2 than on Farm 1, but a confirmatory haematocrit determination was carried out on Farm 2 for every animal judged from the clinical
(FAMACHA©) evaluation to be anaemic. In other words, only on Farm 2 did the evaluator enjoy the advantage of a “Gold Standard” check on every evaluation considered to be anaemic.

The accuracy of the FAMACHA© test in discriminating between alternative states of health at the two evaluated haematocrit cut-offs on Farm 1 was in the moderately accurate range, while on Farm 2 it ranged from moderately to highly accurate at corresponding haematocrit cut-off values (Figs. 1a and b). However, even the smallest area under the curve value (Farm 1 at 0.79) was higher than the accepted minimum value (i.e. 0.7) for a moderately accurate test indicating that testing was accurate even in the absence of calibration against haematocrit – a further confirmation of the robust nature of FAMACHA© testing.

Kaplan et al. (2004), in the southern United States, reported a Se of 1 in sheep when FAMACHA© categories 3, 4 and 5 were considered test positive, at a haematocrit cut-off of ≤15 %. They also found that Se decreased to 0.92 if the haematocrit cut-off was increased to ≤19 %. In South Africa Vatta et al. (2001) reported a Se of 75.7 % and Sp of 55.3 % in goats farmed under communal resource-poor farming conditions, at a haematocrit cut-off of ≤19 % and a FAMACHA© ROC cut point of 3, while for a cut-off of ≤18 % Se and Sp were 80 % and 54.3 %, respectively. The results of Vatta et al. (2001) with goats were broadly similar to the present work involving sheep, where Se on Farm 2 for a cut-off ≤19 %, was found to be 80.9 % for a ROC cut point of 3. The maximum test accuracy for a given haematocrit cut-off value can be read directly off the two-graph ROC plots in Fig. 2a–2d, at the point where the Se and Sp curves intercept (Greiner and Gardner, 2000). However, simply maximising the accuracy, defined as the
point where the Se and Sp curves intersect (Greiner and Gardner, 2000), may not always reflect the epidemiological risk situation for FAMACHA© implementation, since there is always the requirement to maximise Se. Accordingly, the optimum ROC cut point for treatment is set according to the smallest vertical distance between the two curves at a given ROC cut point (or, alternatively, the highest average value for Se + Sp) while still maintaining an acceptable test Se.

TABLE 2. FAMACHA© test ROC cut points and likelihood ratios of a positive test (LR+) for Farm 1 and Farm 2 at haematocrit cut-off values of ≤22 % and ≤19 %. No values are reported for a ROC cut point of 5 on Farm 2, as there were no animals in this category on the farm. (* - haematocrit cut-off values)

<table>
<thead>
<tr>
<th>FAMACHA© ROC cut point</th>
<th>Farm 1; ≤22 % *</th>
<th>Farm 1; ≤19 % *</th>
<th>Farm 2; ≤22 % *</th>
<th>Farm 2; ≤19 % *</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR+</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>(1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(2)</td>
<td>2.25</td>
<td>1.98</td>
<td>2.24</td>
<td>2.07</td>
</tr>
<tr>
<td>(3)</td>
<td>10.47</td>
<td>7.61</td>
<td>9.90</td>
<td>7.35</td>
</tr>
<tr>
<td>(4)</td>
<td>0.94</td>
<td>0.89</td>
<td>49.01</td>
<td>24.03</td>
</tr>
<tr>
<td>(5)</td>
<td>0.99</td>
<td>0.98</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
For Farm 1 test accuracy is maximised at a ROC cut point of 2 (Fig. 2a) and a haematocrit cut-off of $\leq 22\%$, but Se at this cut-off was only 83 %, compared to 93 % at a cut-off of $\leq 19\%$. Thus, for Farm 1, maximising accuracy was rejected in favour of maximising Se without resorting to blanket treatment, and consequently a FAMACHA© ROC cut point of 2 at a haematocrit cut-off of $\leq 19\%$ was recommended. For Farm 2 test accuracy was highest for a ROC cut point of 3, with Se at approximately 80 %, and given the higher overall accuracy of testing on Farm, 2, an ROC cut point of 3 was regarded as a safe treatment threshold.

4.2 Likelihood ratio of a positive test result (LR+) and two-graph ROC analysis

The above findings and our recommendations are further supported by the LR+ (Table 2). Despite the higher level of misclassification on Farm 1, the LR+ for a recommended FAMACHA© ROC cut point of 2 and a haematocrit cut-off of $\leq 19\%$ is 1.98 (Table 2), indicating that an animal randomly selected and scored into this category is on average twice as likely to have a haematocrit of $\leq 19\%$ (and thus being detected as a treatable, anaemic individual) than to have a haematocrit above 19 %. The higher treatment threshold (i.e. FAMACHA© category 2) recommended for Farm 1 compared to FAMACHA© category 3 for Farm 2 was therefore justified by the lower overall LR+ value obtained on the former.

For the more accurate results from Farm 2, the LR+ for the recommended ROC cut point of 3 and haematocrit cut-off of $\leq 22\%$ is 9.90 (Table 2), similarly indicating that an animal selected under this criterion is 9.9 times as likely to have a haematocrit of $\leq 22\%$.
% than to have a haematocrit above 22 %. It is also important to note that, since likelihood ratios are prevalence-independent (Greiner and Gardner 2000), they constitute an objective measure of the likelihood of detecting a diseased animal at any test ROC cut point.

4.3 Results in relation to Targeted Selective Treatment (TST)

The consequences of false negative test results are potentially much more serious than those for false positive diagnoses due to the selective nature of FAMACHA© treatment (Bath et al., 2001). While the latter will lead to some “unnecessary” drenching of non-anaemic animals, the former (i.e. false negative test results) could result in animals being at risk of serious loss in production or even death through not being treated.

The present results are consistent with the main consideration when implementing TST, i.e. that Se should be maximised at the expense of Sp, while still leaving a proportion of the flock undrenched to maintain a sustainable parasite population in refugia (Van Wyk, 2001), especially during periods when the seasonal risk of disease is high, or when there is doubt about the accuracy of testing. For Farm 1 the recommendation to select a FAMACHA© treatment threshold of 2 together with a haematocrit cut-off of \( \leq 19 \% \) will ensure that at this “risky” cut-off an average of 93 % of sheep with a haematocrit of \( \leq 19 \% \) will be detected as diseased and treated, while the total proportion of the animals recommended for treatment throughout the *Haemonchus* season would still only average a maximum of 60 % of the flock, i.e. (176 true positives
+ 227 false positives)/675. This is in contrast to the situation on Farm 2, where, for a $\leq 19\%$ cut-off and treatment threshold of 3, 80% of sheep with a haematocrit of $\leq 19\%$ would be detected, and on average, a maximum of 20% of the flock would be treated (93 true positives + 76 false positives)/806.

The fact that at least at an initial calibration event per farm blood sampling and haematocrit analysis is required for ROC application, makes it relatively labour intensive, and currently not practical for routine on-farm evaluation of animals by farmers. The procedure is not however required routinely as it should theoretically only be necessary to calibrate evaluation against the haematocrit relatively infrequently. The potential advantages of accuracy determination and treatment threshold selection may encourage farmers to adopt the approach despite labour intensity. However, it is particularly in comparative trial work that this method for accuracy determination and treatment threshold selection would be expected to hold most potential.

5. Conclusion

ROC analysis shows strong potential for depicting the trade-off between Se and Sp in situations where, as in the case of haemonchosis, the prevalence of disease is high and there is a significant penalty (i.e. death of an animal in the case of haemonchosis) if an individual with the disease is not detected. The two-graph ROC analysis facilitates objective decisions on appropriate treatment thresholds, which are further supported by the LR+ of positive test diagnoses, and the method appears to be robust to any consistent misclassification by operators.
6. Acknowledgements

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7. References


