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Short communication

FAMACHA© scores history of sheep characterized as resistant/resilient or susceptible to *H. contortus* in artificial infection challenge

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ABSTRACT

With the aim of validating the FAMACHA© as a method for phenotypic selection of sheep resistant/resilient to gastrointestinal nematodes, 27 Suffolk ewes with known FAMACHA© score histories were experimentally infected with 25,000 larvae of *Haemonchus contortus*. From the day of infection (day 0) to 60 days post-infection, at intervals of 7–15 days, ewes were evaluated for packed cell volume (PCV) and fecal egg counts (FEC). A statistically significant increase ($p < 0.05$) in FEC occurred between day 0 and day 60. PCV values showed a decrease ($p < 0.05$) starting from day 21, compared to day 0. Based on the changes in FEC and PCV values from day 0 to day 60, 15 ewes (55.56%) were classified as susceptible (S) and 12 ewes (44.44%) as resistant/resilient (RR). A comparison of the average FEC after infection between susceptible (4487.6 eggs per gram—epg) and resistant/resilient (1317.9 epg) ewes showed a significant difference ($p < 0.05$) between the two groups. The difference in average PCV values after infection (24.8% and 30.3% for S and RR, respectively) was also significant ($p < 0.05$). Data from 980 previous evaluations of FAMACHA© scores from the 27 ewes showed that 58.33% of the ewes classified as RR and 46.67% of the S group had a history of only F1 and F2 scores. In the RR group, only one animal (8.33%) had an F4 score, occurring one time out of the 61 evaluations of this ewe. In contrast, 40.0% of S group ewes had F4 and/or F5 scores. During the period of FAMACHA© score history that was evaluated, 69.56% of the total number of anthelmintic treatments in the flock were administered to ewes from group S. Since ewes with F4 and/or F5 scores during the FAMACHA© score time period were classified as susceptible during the experimental infection (with the exception of one ewe), we conclude that the FAMACHA© score history is a useful tool for the selection of ewes that are resistant/resilient, as well as for the identification of susceptible animals that should be culled.

1. Introduction

Due to the continued development of widespread anthelmintic resistance, control of internal parasites must focus on the use of Integrated Parasite Management (IPM), which combines chemical and nonchemical approaches (Kahn and Woodgate, 2012). IPM includes management practices that minimize infection, strategic deworming of infected and highly susceptible individuals, known as targeted selective treatment (TST), and the genetic selection of lines or breeds of hosts to increase host resistance and/or resilience to parasites (Hoste and Torres-Acosta, 2011).

The best known example of a TST indicator is FAMACHA©, a system developed in South Africa that facilitates the clinical identification of anemic sheep infected with *Haemonchus contortus* by evaluating the ocular mucosa when compared to a standardized chart (Van Wyk and Bath, 2002). In this system of classification, only animals judged to be in categories 3–5 are routinely treated. An important and practical factor is that FAMACHA© does not require laboratory facilities, and has a heritability ranging from 0.24 to 0.55

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(Riley and Van Wyk, 2009), allowing for its use as a characteristic for selection.

The presence or absence of clinical signs in infections caused by *H. contortus* is associated with genetic differences between individuals that characterize animals as resistant, resilient, or susceptible to infections. According to Bishop (2012), resistance to infection essentially describes the host's ability to interact with and control the lifecycle of the parasite, and resilience is defined as the animal's ability to maintain performance in the face of a disease challenge. One of the main benefits of genetically improving resistance to nematodes is the epidemiological effect arising from reduced fecal egg count (FEC). Resistant sheep excrete fewer eggs, leading to reduced larval contamination of pastures. This benefits all animals grazing in the same pasture, regardless of their resistance status (Bishop, 2012). On the other hand, the benefit of having resilient animals in a flock is to preserve refugia, as the presence of resilient animals also results in fewer animals that are identified as individuals that need to be dewormed. Consequently, there will be a reduction in selection pressure for anthelmintic resistance (Kenyon et al., 2009).

The objective of this study was to define the resistance/resilience or susceptible status of ewes artificially infected with *H. contortus* based on blood packed cell volume (PCV) and FEC, and to compare the FAMACHA© score history of these ewes to determine whether the FAMACHA© system is a useful tool in the selection for parasite resistance/resilience.

2. Materials and methods

This study was approved by the Research Ethics Committee of Pontifícia Universidade Católica do Paraná, under the protocol 558-B. Twenty-seven adult, non-pregnant Suffolk ewes, belonging to the Prefeitura Municipal de Curitiba/Paraná/Brazil were evaluated from March to June 2011, after being artificially infected with third-stage larvae (L3) of *H. contortus*. Before the experimental period, all ewes had been analyzed by the FAMACHA© system routinely, as a targeted selective treatment (TST), since 2006. Throughout the experimental period, all animals were maintained in a dry lot, housed on elevated floors, and were fed a 16% crude protein commercial ration, grass hay, and water *ad libitum*.

Pure cultures of *H. contortus* were obtained to perform experimental infections. Ewes to be infected were grazing naturally infected pastures and were treated with levamisole (7.5 mg/kg, Ripercol® L 150F, Zoetis Brazil) and nitroxynil (13 mg/kg, Dovenix Supra®, Merial Brazil) 40 days prior to the infection day (day 0—D0). Ten days after these anthelmintic treatments, the FEC, determined by a modified McMaster's technique (Whitlock, 1948) was below 50 eggs per gram (epg). The ewes were housed in confinement 25 days prior to the D0, when each animal was orally infected with a single dose of 25,000 *H. contortus* L3.

Ewes were evaluated by FEC and blood packed cell volume (PCV) on days (D) 0 (day of infection), 7, 21, 30, 45, 53 and 60 post-infection.

Animals that reached PCV ≤15% were treated with levamisole (7.5 mg/kg, Ripercol® L 150F, Zoetis Brazil) and nitroxynil (13 mg/kg, Dovenix Supra®, Merial Brazil) to avoid possible deaths.

FAMACHA© scores obtained between the years 2006 and 2010 from all animals were tabulated using Excel spreadsheets to determine the frequency and percentage of each FAMACHA© classification (F1–F5) for each ewe during that time period. The number of anthelminthic treatments during this period was also determined.

The classification into resistant/resilient (RR) or susceptible (S) ewe was based on the change in FEC and PCV values from D0 to D60. Ewes that had a decrease in FEC from D0 to D60 were considered resistant; ewes with an increase in FEC and in PCV, or a PCV ≥28% (lower PCV value for classification as F1) at D60 were classified as resilient. Animals meeting either of these criteria were placed in the RR group. Ewes with an increase in FEC and a decrease in PCV from D0 to D60 were considered susceptible and classified within the S group.

The statistical analysis of the average PCV and FEC from the RR and S groups obtained during the experimental infection period was performed using analysis of variance (ANOVA), in a completely randomized design, with two sources of variation (PCV and FEC) in repeated measurements, using the software Statgraphics® Centurion XVI, version 16.1.11. (StatPoint Technologies, Inc., Warrenton, Virginia), followed by Tukey's test with a 5% level of significance. FEC values were log transformed [log 10(x+1)] for the statistical analysis. The data presented in Tables 1 and 2 are the untransformed arithmetic means.

The FAMACHA© scores history was analyzed by descriptive statistics.

Table 1
Mean and standard error of the mean of fecal egg count (FEC), in eggs per gram of feces (epg), and packed cell volume (PCV), in percentage (%), at 0, 7, 21, 30, 45, 53, and 60 days post-infection of 27 Suffolk ewes that were experimentally infected with 25,000 *H. contortus* L3 larvae on day 0.

Days post-infection	FEC (epg)	PCV (%)
0	$1555.6^{ab} \pm 346.5$	$30.8^{c} \pm 0.9$
7	$848.2^{a} \pm 223.5$	$29.9^{bc} \pm 0.7$
21	$1687.0^{ab} \pm 393.7$	$26.3^{ab} \pm 0.9$
30	$4007.4^{bc} \pm 749.5$	$25.1^{a} \pm 0.9$
45	$2896.3^{ab} \pm 519.7$	$26.5^{ab} \pm 0.9$
53	$4266.7^{bc} \pm 941.4$	$25.5^{a} \pm 0.9$
60	$6290.7^{c} \pm 1194.2$	$26.4^{ab} \pm 1.1$
Mean	3078.8 ± 624.1	27.2 ± 0.9

Note: different letters in the same column indicate significantly different values by Tukey's test (5% significance level). FEC values were transformed by log 10(x+1) prior the statistical analysis. Data shown are untransformed arithmetic means.

Table 2
Mean and standard error of fecal egg count (FEC), in eggs per gram of feces (epg), and blood packed cell volume (PCV), in percentage (%), at 0, 7, 21, 30, 45, 53, and 60 days post-infection of Suffolk ewes that were experimentally infected with 25,000 *H. contortus* L3 larvae on day 0, classified into resistant/resilient (RR) or susceptible (S) groups.

Days post-infection	FEC (epg)	
	RR (n = 12)	S (n = 15)
0	$1191.7^{aA} \pm 683.9$	$1846.7^{abA} \pm 2334.0$
7	$512.5^{aA} \pm 461.8$	$1116.7^{aA} \pm 1547.1$
21	$970.8^{aA} \pm 1572.1$	$2260.0^{abA} \pm 2245.1$
30	$1745.8^{aA} \pm 2684.8$	$5816.7^{bcdB} \pm 3822.1$
45	$1408.3^{aA} \pm 1902.5$	$4086.7^{abcB} \pm 2699.0$
53	$1333.3^{aA} \pm 1695.0$	$6613.3^{cdB} \pm 5376.1$
60	$2062.5^{aA} \pm 2389.0$	$9673.3^{dB} \pm 6280.0$
Mean	$1317.9^{A} \pm 1792.0$	$4487.6^{B} \pm 4695.7$
Days post-infection	PCV (%)	
	RR (n = 12)	S (n = 15)
0	$32.6^{aA} \pm 5.0$	$29.3^{cA} \pm 3.6$
7	$31.4^{aA} \pm 3.1$	$28.8^{bcA} \pm 4.1$
21	$28.7^{aB} \pm 3.3$	$24.5^{abA} \pm 5.2$
30	$29.0^{aB} \pm 3.4$	$22.0^{aA} \pm 3.5$
45	$29.9^{aB} \pm 2.7$	$23.7^{aA} \pm 4.0$
53	$29.3^{aB} \pm 3.3$	$22.5^{aA} \pm 3.6$
60	$31.3^{aB} \pm 3.1$	$22.5^{aA} \pm 3.7$
Mean	$30.3^{B} \pm 3.6$	$24.8^{A} \pm 4.8$

Note: different lowercase letters in the same column and different uppercase letters in the same line indicate significantly different values by Tukey's test (5% significance level). FEC values were transformed by log 10(x+1) prior the statistical analysis. Data shown are untransformed arithmetic means.