DETERMINATION OF PARASITE RESISTANCE STATUS TO ANTHELMINTICS THROUGH THE LARVAL DEVELOPMENT TEST

DETERMINAÇÃO DO STATUS DE RESISTÊNCIA PARASITÁRIA A ANTI-HELMÍNTICOS PELO TESTE DE DESENVOLVIMENTO LARVAR

YOUSMEL ALEMÁN GAINZA[1*], RAFAELA REGINA FANTATTO[2], SIMONE CRISTINA MÉO NICIURA[3], ANA CAROLINA DE SOUZA CHAGAS[3]

[1]Faculade de Ciências Agrárias e Veterinárias (FCAV), UNESP, Jaboticabal, SP, Brazil
[2]Faculdade de Ciências Farmacêuticas (FCF), UNESP, Araraquara, SP, Brazil
[3]Embrapa Pecuária Sudeste (CPPSE), São Carlos, SP, Brazil
*e-mail: yousmel@gmail.com

The control of gastrointestinal nematodes of small ruminants has been carried out through intense and indiscriminate use of anthelmintics. However, in the current scenario where resistance to anthelmintics (especially of *Haemonchus contortus*), residues and sustainability are increasingly important elements of production systems, parasite control approaches have changed. Integrated parasite control (IPC) is based on reducing frequency of drug use, integrated with other control measures. IPC is considered a holistic and sustainable approach, with the objective of maintaining/increasing animal production, reducing risks of veterinary drug residues in animal tissues (food safety), reducing environmental contamination (sustainability) and spreading this knowledge to a greater number of producers (equity). Such adjustments have been imposed by technological development itself and by heightened consumer awareness. From this conceptual framework, the purpose of present study was to determine lethal concentrations (LCs) of different anthelmintics for susceptible (McMaster) and resistant (Embrapa2010) isolates of *H. contortus* by *in vitro* tests, establishing dose-response curves. thiabendazole (0.78 to 50 μg.mL^{-1}), levamisole (0.006 to 3.12 μg.mL^{-1}), ivermectin (0.005 to 10 μg.mL^{-1}) and monepantel (0.000975 to 2 μg.mL^{-1}), belonging to different chemical groups, were evaluated in both *H. contortus* isolates by the larval development test (LDT). Feces were recovered from lamb hosts of isolates and eggs were recovered. About 100 eggs were placed in each well of 24-well plates and incubated at 27°C. After 24 h, drug dilutions were inserted into wells containing L_1 and, after 6 days of incubation, L_1, L_2 and L_3 were counted under a microscope to measure the inhibition of larval development by the drugs, and consequently, the parasite level of resistance. Six replicates were performed in three independent experiments for dilutions and for control group (water and culture medium). The results were analyzed using SAS Probit, one-way ANOVA and the Tukey test. For the McMaster isolate, the LC_{50} and LC_{90} values for thiabendazole, levamisole, ivermectin and monepantel were 0.03 and 0.54 μg.mL^{-1}, 0.02 and 0.07 μg.mL^{-1}, 0.07 and 0.33 μg.mL^{-1}, and 0.08 and 0.22 μg.mL^{-1}, respectively. For the Embrapa2010 isolate, the LC_{50} and LC_{90} values were 9.10 and 35.59 μg.mL^{-1}, 0.04 and 0.14 μg.mL^{-1}, 1.61 and 4.62 μg.mL^{-1}, and 0.29 and 0.72 μg.mL^{-1}, respectively. All drugs had a dose-dependent response and statistically different effect ($p \geq 0.05$) between the isolates. The efficacy of the drugs in inhibiting the larval development, as well as the LCs obtained, confirmed the susceptibility and resistance status of McMaster and Embrapa2010, respectively. We intend in the future to submit parasites collected from farms to the LDT and compare their response to the drugs in relation to the two extreme isolates. Thus, the resistance status of parasites of these farms would be known and monitored for the four chemical groups. It would be possible to recommend the most effective drug for a particular farm, reducing the need for deworming and rationalizing anthelmintic use.

Keywords: *Haemonchus contortus*, resistance diagnosis, sheep.

Acknowledgments: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP), Embrapa.