



ANTHELMINTIC RESISTANCE DIAGNOSIS OF *Haemonchus contortus* ISOLATES THROUGH RESISTA-Test[®]

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A novel larval development test (LDT) named RESISTA-Test[®] was developed to detect anthelmintic resistance using a 96-well culture plate. The RESISTA-Test[®] detects the efficacy of thiabendazole (TBZ), ivermectin (IVM), levamisole (LEV), and monepantel (MPT) and was used to confirm the susceptibility or resistance in two *Haemonchus contortus* isolates (*Echevarria1991 - HcEc91*, considered susceptible and *Botucatu - HcBot*, considered resistant). Each isolate was kept in eight lambs that grazed in separate paddocks (experimental lambs) for 160 days post artificial infection. Faecal samples of two donor lambs (used as reference to calculate the resistance factor) infected with *HcEc91* and two donor lambs infected with *HcBot* were collected and analysed. Eggs recovered from the animals' faeces were incubated at 27°C with the chemical groups and culture medium for seven days to observe L₃ development. The RESISTA-Test[®] results were analysed using logit dose-response by the Probit model. The degree of parasitic resistance for each anthelmintic was expressed by the resistance factor (RF: calculated as the LC₅₀ values of the studied isolate divided by the respective susceptible isolate values). For the *HcEc91* isolate in the experimental lambs, the RF values were TIA = 0.7, IVM = 0, LEV = 1.1 and MPT = 0.9. The RF values for the *HcBot* isolate in the donor lambs were TIA = 147, IVM = 176, LEV = 931, and MPT = 53 and for the experimental lambs the RF values were TIA = 141, IVM = 196, LEV = 822, and MPT = 34. The results confirmed with accuracy the anthelmintic resistance status of the two isolates since the RF values for the susceptible isolate were below 3 and the contrary was observed for the resistant isolate, with RF values above 3. Moreover, RF values for the donor and experimental lambs infected with *HcBot* isolate were quite similar for all chemical groups demonstrating that both isolates can be kept successfully in the field as long as lambs graze different paddocks. The results demonstrated that RESISTA-Test[®] can be used in the laboratory routine, to detect in a simple way the anthelmintic resistance status of *H. contortus* without the need of applying unnecessary anthelmintic treatments such as those used to perform faecal egg count reduction test (FECRT). This tool would be adopted to support the farm's parasite control programs, enabling a more targeted, efficient, and sustainable gastrointestinal nematode control.

Keywords: gastrointestinal nematodes; ovine; *in vitro*.

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