DETECTION OF BACTERIAL CONTAMINANTS BY THE LARVAL DEVELOPMENT TEST

DETECÇAO DE CONTAMINANTES BACTERIANOS NO TESTE DE DESENVOLVIMENTO LARVAR

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The resistance diagnosis of gastrointestinal nematodes of sheep and goats to commercial anthelmintics is an important tool to guide farmers about the use of the most effective dewormer in their flocks, promoting sustainable production. There are several *in vitro* tests that are available to detect resistance to anthelmintics. They are based on exposure of the parasite (eggs, larvae and adults) to different anthelmintic groups in culture dishes. After incubation, the parasites are counted and the drug efficacy is calculated in function of inhibition of parasitic cycle or mortality. The larval developmental test - LDT (L_1 to L_3) and egg hatch test - EHT are the most common techniques used for *in vitro* diagnosis. In both, it is crucial for either the parasites to be incubated or the culture plates to be free of contaminants. The HACCP (Hazard Analysis and Critical Control Points) system has been a useful tool to assess risks and establish control measures to minimize the risks of physical, chemical or microbiological contamination. The use of some principles of the HACCP system, such as the traceability of a contaminant, is a useful tool in laboratory protocols and processes. It is an alternative in the selection of critical control points (CCPs), enabling the eradication or control of most pathogens, especially those that cause problems at important research stages. Thus, the purpose of this study was to identify the agents causing high larval mortality that were detected in negative control groups of the LDT. In this control (no treatment with anthelmintics), inhibition of larval development was higher than the accepted limit (10%). Twenty-five samples were collected by swabs from all materials and reagents used for recovery of *Haemonchus contortus* nematode eggs (isolated from host feces) and from materials used in the LDT itself. The collected material was seeded in plates with blood agar media and incubated for 24 h at 37°C. Cultures that were positive (presence of colonies) were submitted to the gram staining test for physicochemical identification of their cell walls. The main results obtained in the cultures were negative for: 1) sieves for egg retention; 2) glassware; 3) distilled water collectors and 4) the BOD chamber for parasite incubation. However, growth of microorganisms was detected in 50 mL Falcon tubes (used to recover and suspend eggs after centrifugation), suggestive of *Pseudomonas* spp. and *Staphylococcus aureus*, identified by gram staining and observation by optical microscopy (2000X). Thus, it was possible to verify that the recovery process of eggs from the fecal material is one of the most critical points of the LDT, being responsible for the contamination of *in vitro* tests and impairing the diagnosis of anthelminthic resistance. Therefore, this step should be carefully monitored for the presence of biological contaminants.

Keywords: diagnosis, HACCP , *in vitro* tests.

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