USE OF CALCOFLUOR WHITE AND IMAGE ANALYSIS FOR QUANTIFYING TRICHOSTRONGYLE EGGS FROM SHEEP FECAL SAMPLES

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ABSTRACT

The most commonly used method for estimating intestinal parasite loads in sheep and cattle consists of counting the eggs isolated from feces with a floatation concentration method. The most labor intensive portion of this process is actually counting the eggs observed with a microscope. Image analysis software can be used to instantaneously count the eggs if the software can correctly identify these eggs apart from the contaminating fecal debris (mostly plant material). Calcofluor is a commonly used fungal stain, which binds to chiton and other exo-polysaccharides, has not been evaluated for use as stain for trichostongyle eggs. For this study, trichostrongyle eggs (mostly Haemonchus contortus) were isolated from naturally-infected sheep fecal samples using the Wisconsin sucrose floatation procedure. Eggs were stained by suspending the centrifuged fecal pellet into 3-4 ml of 0.5% (w/v) calcofluor for 20 minutes, followed by re-centrifugation and sucrose floatation. Floated eggs were captured on a 22X22mm coverslip, observed with an AX70 Olympus microscope equipped for UV epi-fluorescence and photographed using an Olympus DP-70 digital camera. Unstained eggs exhibited low-level auto-fluorescence which was generally lower than that of the contaminating fecal debris. Calcofluor-stained eggs fluoresced intensely at a level that was 40-80 times the auto-fluorescence of the unstained eggs. Exposure-times selected by the camera using the spot-meter system eliminated much of the auto-fluorescence from the fecal debris. The use of Calcofluor should greatly improve the ability of image analysis software to correctly identify trichostrongyle eggs from the contaminating fecal debris, and possibly speed up the quantification of these eggs in sheep and cattle fecal samples.