Fasciola spp. faecal sedimentation protocol for concentration of eggs and DNA isolation Version 2

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Abstract

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Protocol

Traditional sedimentation for Fasciola spp.

Step 1.
Mix faecal samples (3 g and 6 g for sheep and cattle, respectively) with distilled water to form a homogenous solution.

Traditional sedimentation for Fasciola spp.

Step 2.
Hose the solution through a 270 µm nylon sieve into a 250 ml conical measuring cylinder, top with distilled water and allow to sediment for three minutes.

Traditional sedimentation for Fasciola spp.

Step 3.
After three minutes aspirate the supernatant and pour the sediment into a 100 ml conical measuring cylinder, rinse the 250 ml conical cylinder into the new cylinder and top with distilled water. Allow to sediment for a further three minutes.

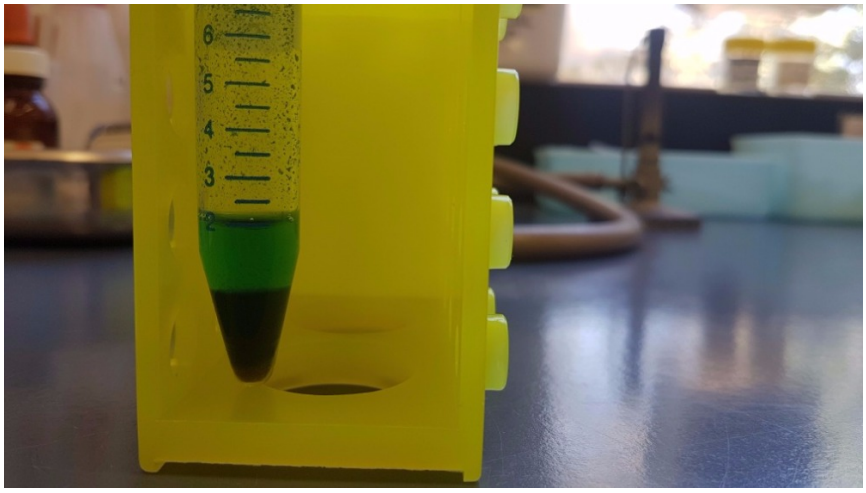
Traditional sedimentation for Fasciola spp.

Step 4.
Aspirate the supernatant and pour the remaining sediment into a 15 ml centrifuge tube, rinse the 100 ml conical cylinder into the 15 ml centrifuge tube and top with distilled water. Allow to sediment for a final three minutes.

Traditional sedimentation for Fasciola spp.

Step 5.

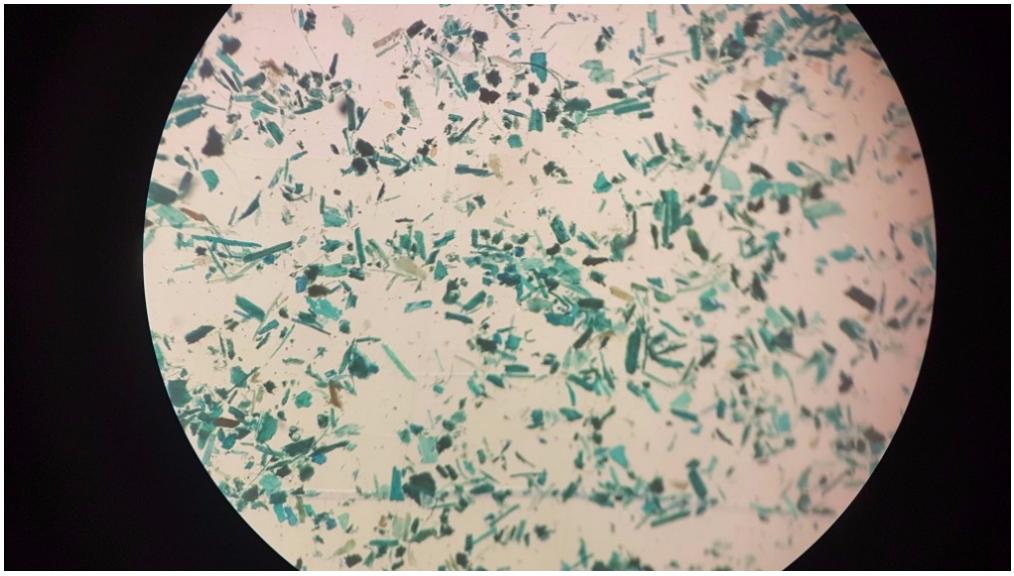
Aspirate the supernatant, leaving 2 ml of sediment. To perform a faecal egg count proceed to step 6. To go straight to DNA isolation proceed to step 9.



Faecal egg count (FEC) for EPG calculation

Step 6.

To examine the sediment for fluke eggs, add 2 drops of methylene blue (1%), shake to mix and rinse into a 6.5×17×1 cm grid perspex tray. Additional distilled water can be added to allow for ease of counting. Examine under a stereomicroscope at 15× magnification.



Faecal egg count (FEC) for EPG calculation

Step 7.

All yellow-brown Fascola eggs should be counted. Counts should be divided by 2 to calculate eggs per gram (EPG) for cattle. EPGs for sheep are as observed.

Concentration for disruption and DNA isolation

Step 8.

If samples were used to calculate EPG: Return individual samples to the 15 ml centrifuge tubes and centrifuged at 2500 g for 10 minutes to form a pellet.

If proceeding straight from step 5: Centrifuge samples at 2500 g for 10 minutes to form a pellet.

Concentration for disruption and DNA isolation

Step 9.

Manually remove the entire pellet from the 15 ml centrifuge tube using a combination of Pasteur pipettes and fine wooden applicator sticks and place into a pre-prepared bead-beating tube containing ceramic beads and lysis buffer (BioLine Isolate Fecal DNA Kit).

Concentration for disruption and DNA isolation

Step 10.

Disrupt the samples at 6.0 m/s for 40 seconds on a bench top homogeniser (FastPrep®-24 MP Biomedicals, Australia). Place on ice after disruption until continuing with DNA isolation (BioLine Isolate Fecal DNA Kit) and amplification.