DETECTION OF *Fasciola hepatica* IN FIRST STAGES BY COPROLOGICAL, MOLECULAR AND IMMUNOLOGICAL METHODS.

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The diagnosis of fasciolosis is based on coprological methods from 8 weeks post infection (pi). Because of the low sensitivity of this technique, the aim of this study was to evaluate molecular and immunological techniques in faecal samples.

Faeces were collected weekly from sheep experimentally infected by *F. hepatica*, until 8 weeks pi. Moreover, 27 samples were taken randomly from a naturally infected flock and analyzed by sedimentation, PCR and direct-ELISA.

After carrying out a preliminary PCR with specific primers, samples taken from 1-2 weeks pi were negative so a nested-PCR was designed using firstly general primers, and then specific primers. These PCRs were also used to diagnose the infection in the naturally infected flock. On the other hand, we carried out a commercial direct-ELISA kit (Bio-x) with all faecal samples.

By sedimentation, *F. hepatica* eggs were not detected in the experimental animals until 8 weeks pi; in the naturally infected flock, 6 out of 27 samples were positive. After PCRs, the diagnosis of fasciolosis in the experimentally infected group was carried out between weeks 3-8 pi by PCR with the specific primers and amplifying a 424 bp band. By means of the nested-PCR, the same band was detected in samples collected on week 2 pi. In naturally infected animals, all faecal samples were positive by nested-PCR. On the other hand, the direct-ELISA detected the infection from week 4 pi in the experimentally infected group and in all samples from the naturally infected flock.

In conclusion, the infection could be detected by a PCR in faeces from the second week pi and by a direct ELISA from the fourth week. Moreover, both methods are more accurate to diagnose the infection under field conditions than sedimentation.

**Palabras clave:** *Fasciola hepatica*, diagnosis, sedimentation, PCR, direct-ELISA