

# Abstract

**Charles University**

**Faculty of Pharmacy in Hradec Králové**

**Department of Biochemical Sciences**

**Candidate:** Bc. Kristýna Bezdíčková

**Supervisor:** doc. Ing. Petra Matoušková, Ph.D.

**Title of diploma thesis:** Implementation of the diagnosis of gastrointestinal nematodes using RT-PCR

Gastrointestinal nematodes can cause various diseases in farm animals. Individuals are often infected with several types of parasites at once. Since the symptoms tend to be similar, the disease is called parasitic gastroenteritis. Not only do nematodes threaten the animal health, but they can also have a major impact on the economy of the entire farm. Diagnosis is not always easy. In addition to morphological and coprological methods, biochemical or molecular analysis can be used, which tends to be more sensitive and accurate.

We tried to diagnose gastrointestinal nematodes using the molecular RT-PCR method. In the thesis we focused on 6 species of nematodes (*Haemonchus contortus*, *Teladorsagia circumcincta*, *Trichostrongylus spp.*, *Nematodirus battus*, *Chabertia ovina* and *Ashworthius sidemi*). The material used was faeces samples from sheep from which we isolated gDNA. The control material was gDNA isolated from *H. contortus* eggs and gDNA obtained from whole parasite bodies. RT-PCR was tested using the fluorescent dye SYBR Green I and TaqMan probe. The results of the analysis using the dye SYBR Green I and the TaqMan probe were correlated. However, in the cross-reactivity test, the Hco ITS2 primers designed for *H. contortus* also amplified the gDNA of other parasites. The other primers mainly amplified the gDNA of the nematode for which they were designed. In addition to the Hco ITS2 primers, the reference genes GAPDH, NCBP and FARB were also tested, but turned out to be unsuitable for this type of determination.