

A novel diagnostic PCR for the gastro-intestinal nematode *Ashworthius sidemi*

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The blood-sucking gastro-intestinal (GI) nematode *Ashworthius sidemi* is present in deer populations and herds of *Bison bonasus* in Poland. The parasite is considered to be as pathogenic as *Haemonchus* sp., consequently making it an important pathogen in wildlife and domesticated animals. Coproscopical examination of eggs and infective larvae is not sufficiently accurate to differentiate *A. sidemi* from other common GI nematodes in ruminants. Furthermore, prevalence of *A. sidemi* has been reported from a number of neighbouring countries still it is plausible to expect that *A. sidemi* is an underreported parasite in wild and domesticated animals in Europe.

A species-specific PCR method was developed for the detection of *A. sidemi* DNA. DNA was isolated from adult worms obtained from bison abomasums. The PCR is based on a fragment spanning over the 3'-end of *ssrRNA* to the 5'-end of *lsrRNA* (Genbank accession: EF467325). The species-specific primers are directed to unique sequences in the internal transcribed spacer (ITS)1 and ITS2 generating a 406 bp fragment. The specificity of the designed primer-pair was evaluated using template DNA from closely related nematodes in the family Haemonchidae. The highest achieved sensitivity in heterologous DNA samples resulting in positive PCR runs was 10 pg genomic DNA. In conclusion, we have developed a molecular tool that provides species-specific identification of *A. sidemi*. The achieved levels of specificity and sensitivity are adequate for epidemiological studies of the parasite. The novel PCR has also been included in a multiplex PCR assay capable of detecting four important GI species simultaneously.