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New methods for the detection of anthelmintic resistance

A key component of the strategies that will be developed by PARASOL is the measurement of anthelmintic efficacy and of the prevalence of resistance alleles in animals/herds to be treated. Treatment with agents that will obviously be ineffective at killing worms will waste resources and is likely to contribute to unnecessary residues and environmental contamination. Detection of resistance alleles before they reach a level that causes obvious clinical failures is an important method for maximising the sustainability of current drugs.

At present, analysis of the susceptibility of a nematode population against an anthelmintic can be performed by testing adult or larval worm stages *in vivo* or *in vitro*, though *in vitro* tests are not available for all anthelmintics and all species of parasite. The most often used *in vivo* test, the faecal egg count reduction test (FECRT), requires two samplings of at least 12 animals and microscopic examination of the samples, thus causing considerable costs. Of the *in vitro* tests, the egg hatch inhibition test (EHT) has been shown to be suitable for the analysis of benzimidazole (BZ) resistance in a wide range of nematode species of humans, small ruminants, horses and pigs. The concentration of an anthelmintic at which, compared to the controls, 50% (Egg death 50 - ED50) of the larvae are inhibited from hatching is taken as indicator for the level of resistance. However, no *in vitro* has been described for ML resistant cattle nematode species and thus there remains a pressing need for a reliable test for an *in vitro* ML resistance test for cattle nematodes. Tests based on the inhibition of larval movement by MLs, like the micromotility or larval migration tests will be developed for this purpose.

However, such *in vitro* tests may only detect the presence of resistant nematodes once they form a large proportion of the population (25%), though the adoption of discriminating doses (WP3) may improve this. Molecular tests are required to detect the presence of resistance before it becomes a clinical problem, and to prevent it becoming one. Until now, molecular techniques have only been described for BZ resistance in gastrointestinal nematodes of ruminants, due to the fact that knowledge on the molecular mechanism of resistance is confined to the BZs. Several allelespecific polymerase chain reaction methods for the genotyping of different nematode species have been reported. These molecular systems are much more sensitive than the *in vitro* tests described above, detecting down to 1% of resistant individuals. Recent improvements and extensions of the PCR technology like quantitative real-time PCR and the Pyrosequencing technology now offer promising new opportunities to develop molecular test applicable for routine field testing. Pyrosequencing™ is a method suitable for quantitative SNP detection based on a sequencing-by-synthesis reaction and provides reproducible and exact quantifications of single nucleotide polymorphisms. By using these techniques, in the first two years of the project quantitative allele-specific test systems suitable for analysing DNA samples obtained of samples with representative numbers of

pooled worms were established. Sequence polymorphisms related to the ML resistance phenotype have been evaluated with respect to their role as markers or indicators for the resistance level during. New molecular tests have been developed and will now be used to test field samples obtained during evaluating the efficacy of common drugs in four countries. In this way the available molecular data on the mechanism of BZ resistance of gastrointestinal nematodes will be exploited and regarding ML resistance knowledge obtained within the present project is directly applied.