Characterization of non-specific resistance mechanisms against ivermectin in *Cooperia oncophora*

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Background: While facing increasing anthelmintic resistance, knowledge about resistance mechanism becomes crucial. Despite its importance, for resistance to macrocyclic lactones this still remains unresolved. Non-specific mechanisms of resistance involving transporters, e.g. P-glycoproteins (Pgps), or inactivating enzymes, e.g. Cytochrome P450s (CyP450s), are believed to play an important role. Nematodes have a complex repertoire of both protein classes. It is currently unknown, which transporter or enzyme system is associated with resistance to certain anthelmintics. The cattle nematode *Cooperia oncophora* was used as a model to study contribution of both, CyP450s and two individual Pgps, to the development of resistance in more detail.
Methods: The response of susceptible and ML-resistant isolates of *C. oncophora* to ivermectin were evaluated *in vitro* in the presence of Pgp and CyP450-inhibitors verapamil hydrochloride (VPL) and piperonyl butoxide (PBO), respectively,. Full-length Pgp cDNAs were cloned following amplification by RT-PCR and RACE-PCR. Sequence variation of two Pgps was compared between six *C. oncophora* isolates with different resistance status by SeqDoc-analysis. Inducibility of Pgp transcript level by ivermectin was analysed by real-time RT-PCR.
Results: In the presence of VPL and PBO the susceptibility of all tested isolates to ivermectin was significantly increased in the *in vitro* assays measuring development (100-fold) or motility (10-fold). Comparison of full length Pgp-2 and Pgp-3 (currently 65% of the sequence analysed) sequences between *C. oncophora* isolates revealed no major differences correlating with resistance though sequence variability was generally lower in resistant isolates. Preliminary results indicate only minor differences in Pgp-2 expression levels between *C. oncophora* isolates. Expression of Pgp-3 and inducibility of both Pgps by ivermectin are currently examined.
Summary/Conclusions: Though these data indicate that degradation and extrusion as non-specific xenobiotic detoxification mechanisms are both involved in ivermectin-resistance in *C. oncophora*, identification of the relevant genes still remains problematic. The characterised panel of *C. oncophora* isolates nevertheless provides a useful tool for further analyses of specific Pgp and CyP450 genes and their possible contribution to ivermectin efflux and metabolism.