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FELLOWSHIP FINAL REPORT

Drug efflux-mediated processes of anthelmintic resistance in ascarids

Georg von Samson-Himmelstjerna[2,4], Abdallah Harmache[1], Oceane Lénhof[1], Jürgen Krücken[2], Alexandre Vernudachi[3], Cédric Neveu[1]

[1]UMR1282 Infectiologie et Santé Publique, INRAE, Nouzilly, France;

[2]Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany;

[3]INVENesis France Sàrl, Tours, France

[4]LE STUDIUM Institute for Advanced Studies, 45000 Orléans, France

REPORT INFO

Fellow: **Georg von Samson-Himmelstjerna**
From Institute for Parasitology and Tropical Veterinary Medicine, Freie Universität Berlin, Berlin, Germany
Host laboratory in region Centre-Val de Loire: UMR1282 Infectiologie et Santé Publique, INRAE, Nouzilly, France
Host scientist: Dr. Abdallah Harmache
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ABSTRACT

Helminth infections represent a major health threat for both humans and animals. In the latter they occur at often very high prevalences and on a global scale. Due to a near complete lack of immuno-prophylactic measures the metaphylactic use of chemotherapeutics i.e. the anthelmintics is the corner stone of worm control since decades. This has resulted in widespread anthelmintic resistance in a range of helminth species. Particularly the gastrointestinal nematodes and amongst them also so called roundworms or ascarids have evolved resistance. This results in an increasing clinical issue as like in horses the Parascaris spp. as well as in pigs or humans the Ascarais spp. often cause considerable clinical symptoms. To improve the sustainable use and provide solutions for the resistance problem it is important to understand the molecular mechanisms of anthelmintic resistance. In the present project the P-glycoprotein (Pgp) based drug efflux as a non-drug target associated mechanism of resistance is being addressed in Parascaris. To this end, the model nematode Caenorhabditis elegans was employed and the specific role of the Pgp3 was examined. The pharmacological profile of a Pgp3 knock out C. elegans line (VC 2338) was established using a panel of anthelmintic compounds and compared with the N2 wildtype strain. Furthermore, the Parascaris Pgp3 coding sequence was injected into the VC2338 to achieve recombinant expression under an intestinal promotor (ges1) as we have done it successfully with another Parascaris Pgp recently. Our results suggest that the Parascaris Pgp3 overexpression in C. elegans interferes with the development of the worm and ongoing investigations attempt to further elucidate the specific role of Pgp3 in the worms homoeostasis.

1- Introduction

Infections with gastro-intestinal tract helminths parasites occur in all animal species, potentially leading to significant impairment of the development and health of infected individuals. In animals, infections with gastro-intestinal-nematodes (GIN) are of highest relevance as it affects each and every grazing animal. The largest GIN are roundworms/ascarids which can grow up to 50 cm in length and residing in the small intestine not only deprive the host from nutrients but in case of high infection intensities can also lead to intestinal obstruction. This also applies to ascarid infections in man, which are considered to occur in approx. 800 million globally, of which especially children are most often affected. In the absence of any vaccine for protection, treatment and control of ascarid infections heavily relies on the application of anthelmintic drugs. As commonly known also in bacterial

infections this has led to the development and spread of drug resistance in GIN. For example in the horse ascarid *Parascaris univalens* resistance has been reported for all available anthelmintic drug classes, lately also including benzimidazoles (BZ). However, also in humans, where BZs are the only used anthelmintics in mass-drug-application campaigns performed in endemic countries, there is concern of loss of efficacy of this drug class. It is thus of highest One Health relevance to improve our currently poorly developed understanding of BZ resistance mechanisms in ascarids. In the present collaboration researchers from Freie Universität Berlin, Germany and INRAE Nouzilly, France joined to explore new scientific and experimental avenues to examine BZ efflux mediated processes in ascarids by heterologous expression using the model nematode *Caenorhabditis elegans*. The focus was on the role of the P-glycoproteins (Pgp) which as transmembrane efflux pumps have previously been intensively studied already for example concerning their involvement in the resistance against macrocyclic lactones in nematodes and in few investigations also suggested to contribute to BZ resistance. However, the functional analysis of Pgp mediated BZ transport characteristics has thus far not been performed though it is important for the better understanding of drug metabolism in nematodes and thus putative resistance pathways.

2- Experimental details

To study the potential function of *Parascaris* Pgps in BZ efflux we focussed in this project on the Pgp3 as this has recently been identified to be potentially associated with BZ-resistance in the sheep nematode species *Haemonchus contortus* (Kellerova et al. 2020). The *C. elegans* Pgp3 knock out line VC2338 was chosen for the heterologous expression of the respective *Parascaris* Pgp. The pharmacological phenotype of the *C. elegans* knock out strain was characterized in comparison to the N2 wild type by establishing EC_{50} values in an automated and standardized Larval Development Assay (LDA) using a set of eight anthelmintics derived from representing the BZ, ML as well as the cyclooctadepsipeptide emodepside and the tetrahydropyrimidine pyrantel.

For the heterologous expression of the *Parascaris* Pgp3 a plasmid was constructed by which the Pgp3 is being expressed under the control of the intestinal promotor Ges1 similarly to how it has been done with another *Parascaris* Pgp in an earlier collaboration of the fellow's and host's research groups (Gerhard et al. 2020). As a selectional marker the expression of mCherry und the neuronal ODR promotor was chosen (Figure 1).

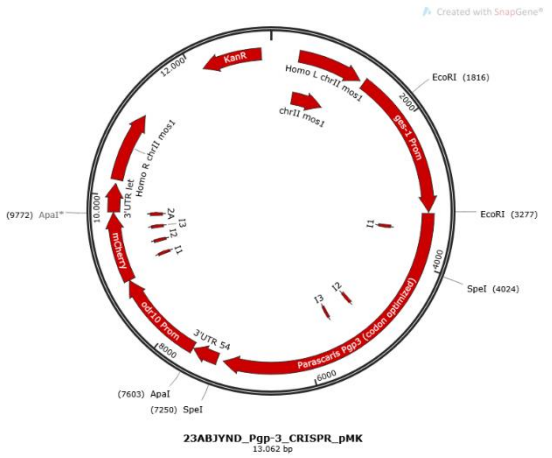
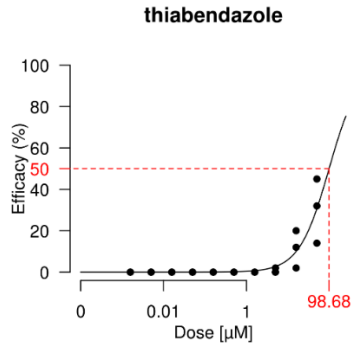


Figure 1 : Map of *Parascaris* Pgp3 expression and mCherry reporter construct.

3- Results and discussion

Surprisingly, in the comparative LDA analysis using the wildtype *C. elegans* N2 strain and the VC2338 line an unexpected significantly lower susceptibility was observed in the VC2338 line for thiabendazole (Figure 2).

A



B

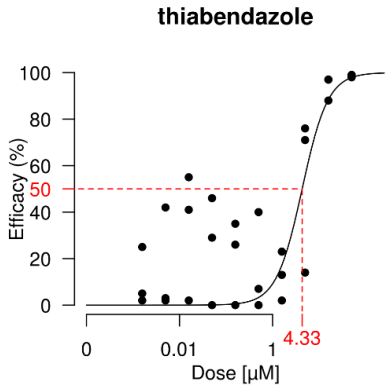


Figure 2: Efficacy concerning the inhibition of Caenorhabditis elegans larvae at different concentrations of thiabendazole and calculated concentration with 50% inhibition (provided in red) for the N2 (A) and the VC2337 (B) strains.

Initial experiments using a 50 ng/µl concentration of the construct for *C. elegans* microinjections did not result in the development of fluorescent offspring. In contrast following enzymatic excision of parts of the Pgp3 sequence from the construct using EcoRI, mCherry expressing offspring were obtained, indicating the unexpected presence of *Parascaris* Pgp3 toxicity in *C. elegans* (Figure 3).

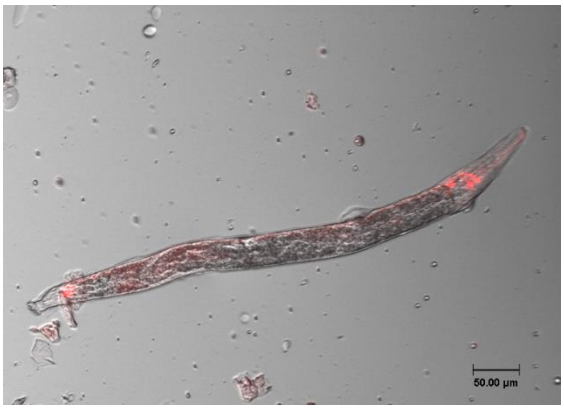


Figure 3. *Caenorhabditis elegans* N2 worm expressing mCherry in head and tail neurons following injection of EcoRI digested *Parascaris* Pgp3 expression construct.

4- Conclusion

Further experiments will now aim at heterologous expression of the *P. univalves* Pgp3 in *C. elegans* N2 employing a new strategy circumventing the putatitvely toxic effects of overexpression of *Parascaris* Pgps.

5- Articles published in the framework of the fellowship

During this fellowship a joint publication building on a previous Le Studium supported collaboration between the fellow and host together with a range of other international scientist was finalized and successfully published (Wolstenholme et al. 2023)

6- Acknowledgements

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