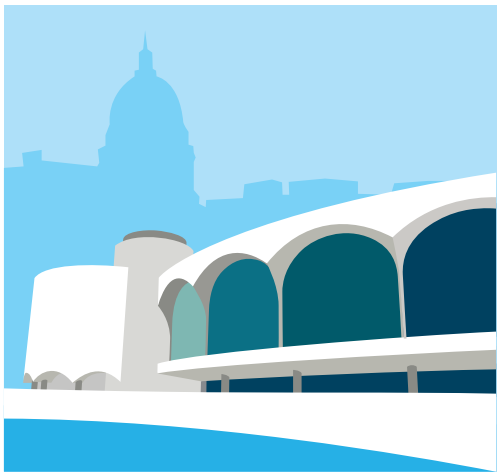
Effect of macrocyclic lactones on Parascaris sp. glutamate-gated chloride channels

Nicolas Lamassiaude, Elise Courtot, Cédric Neveu, Claude Charvet



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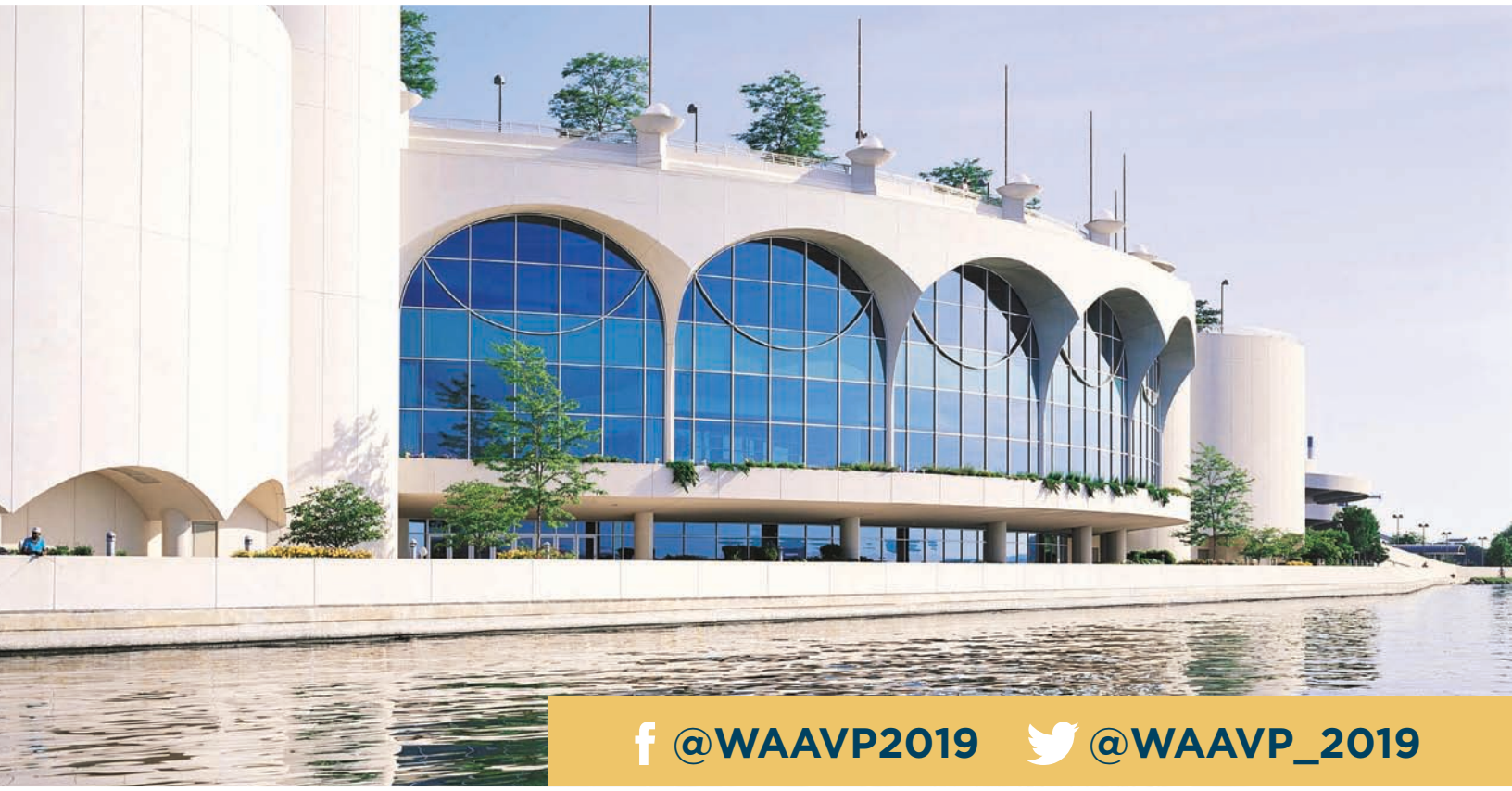

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Abstract Book

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is an increasing threat to equine welfare as resistance to all available drug classes have been found in recent years. Despite this fact, the genetic and molecular background of drug metabolism and resistance in P. univalens is still largely unknown. The aim of this study is to gain deeper knowledge about drug metabolizing genes in P. univalens. To achieve this a whole genome approach was applied, which is an unbiased and systematic method to identify genes involved in different pathways responding to anthelmintic treatment. We have used RNA-Seq to compare the transcriptomes of adult P.univalens after in vitro exposure to three different drug classes.

Adult P.univalens was obtained after slaughter from two 6-month old Icelandic horses that had never been treated with anthelmintic drugs. In the laboratory the worms were in vitro exposed to ivermectin, thiabendazole and pyrantel, each at three different concentrations, for 24 h. RNA was extracted from the anterior end of the parasite and three biological replicates per concentration were sequenced using Illumina NovaSeq.

Sequencing of 36 samples (27 exposed and 9 controls) generated 20,462,622 to 35,338,763 paired-end reads with a length of 100bp per sample. A mapping index was created using the previously published genome (GenBank: NINM00000000.1), transcript expression were quantified using Salmon and differential gene expression analysis was performed using R package DESeq2.

Preliminary results show that between 35 and 474 genes are significantly differentially expressed ($p < 0,05$) in response to exposure depending on drug and concentration. Ongoing analysis of KEGG pathways will provide insights about the function of these genes and should help us gain more understanding of drug metabolism in P.univalens.

OA48.05 Effect of Macrocyclic Lactones on Parascaris Sp. Glutamate-Gated Chloride Channels

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Parascaris sp. is the largest parasitic nematode of horse causing digestive and respiratory disorders to the animal. The control of equine ascaridiosis relies on anthelmintic treatments including macrocyclic lactones (MLs) as the gold standard. However, control of infestation is increasingly difficult due to the emergence of resistant parasites throughout the world. In the free-living model nematode Caenorhabditis elegans, glutamate-dependent chloride channel receptors (GluCls) were identified as the main targets of MLs. However, in Parascaris sp, the mode of action of MLs remain poorly understood. Here we identified the Parascaris sp. GluCls and characterized the effect of a wide range of MLs.

Using a candidate gene approach, we identified the orthologs of 6 genes encoding GluCls subunits in Parascaris sp. The complete cDNAs encoding these subunits were amplified by PCR and cloned into a transcription vector. The corresponding cRNAs were synthesized in vitro and then microinjected into Xenopus laevis oocytes. Two-electrode voltage-clamp experiments were performed on recombinant GluCls to investigate their pharmacological properties. Thus, the expression of a single subunit and combination of different subunits in Xenopus oocytes allowed us to obtain the functional homeric and heteromeric GluCls of Parascaris sp. The receptors were both sensitive to glutamate and ivermectin but the effect of seven different ML compounds revealed striking differences. The physiological function and the impact of MLs on these receptors in vivo are in progress.

This study, provides a better understanding of the pharmacology of GluCls as well as the mode of action of MLs in nematode parasites.