GHENT UNIVERSITY

FACULTY OF VETERINARY MEDICINE
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INVITATION PUBLIC DEFENSE

Role of glycosylation in the immunogenicity of the nematode specific activation-associated secreted proteins (ASPs) and its implication in vaccine efficacy.

Laurens Zwanenburg
10-01-2024 at 17:30h

PROMOTORS
Prof. dr. Peter Geldhof
Faculty of Veterinary Medicine, UGent

Dr. Leen Seys
Faculty of Veterinary Medicine, UGent

Curriculum vitae

Laurens Zwanenburg was born on October 2nd, 1993, in Rotterdam, the Netherlands. In 2012, he relocated to Belgium to pursue a bachelor's degree in veterinary medicine at the University of Antwerp, graduating with distinction in 2015. He continued his education at Ghent University and as part of the master's thesis, he participated in an Erasmus+ program at the University of Copenhagen in 2018. In that same year, he graduated at Ghent University, earning a master's degree in veterinary medicine (major in Research) with great distinction.

Driven by his interest in parasitology, Laurens embarked on a PhD journey in veterinary parasitology, for which he obtained a PhD fellowship awarded by the Fonds voor Wetenschappelijk Onderzoek – Vlaanderen (FWO). During this PhD, Laurens worked on the development of a vaccine against two gastrointestinal parasites in cattle, in collaboration with research groups from Leiden University Medical Center and Wageningen University and Research.

Throughout his PhD, Laurens has generated 1 scientific publication in a high-impact journal and actively engaged in three national and international conferences.

Where?

The defense will take place on the 10th of January 2024 at 17.30h

Clinic Auditorium A (entrance 12 – first floor)

Faculty of Veterinary Medicine, Ghent University

Salisburylaan 133, Merelbeke

https://www.ugent.be/nl/univgent/campussen/campussen-gent/grondplan_campusmerelbeke.pdf

The presentation will be in English.

How to attend?

Please feel free to attend the public defense.

If you would like to join for food and drinks after the defense, please register before 24-12-2023, by email to Laurens.Zwanenburg@UGent.be

Members of the Jury

Prof. dr. Herman Favoreel

Chairman of the Jury

Faculty of Veterinary Medicine, UGent

Prof. dr. Eric Cox

Faculty of Veterinary Medicine, UGent

Dr. Katrien Claes

Flemish Institute for Biotechnology

Faculty of Sciences, UGent

Prof. dr. Benjamin G. Dewals

Faculty of Veterinary Medicine, ULiège

Dr. Alasdair Nisbet

Moredun Research Institute

Summary

The first chapter of this doctoral thesis provides an introduction to *Ostertagia ostertagi* and *Cooperia oncophora*, two gastrointestinal nematodes that hold significant economic importance in the cattle industry. Nearly every animal is either infected or at risk of being infected and thus these parasites pose a risk to animal health, welfare, and, in the case of farm animals, productivity. The primary approach to control these nematode infections involves a regular administration of anthelmintic drugs. However, a control strategy that depends solely on anthelmintics for the long term is unsustainable, given the growing global concern of anthelmintic resistance. Considering sustainability and cost-effectiveness, vaccination offers a promising alternative for the control of nematode infections. Unfortunately, advancement in vaccine development have been limited. In the case of *O. ostertagi* and *C. oncophora*, immunisation of calves with activation-associated secreted proteins (ASP) obtained from adult worms, Oo-ASP-1 and Co-dd-ASP respectively, resulted in protection against the respective parasites. However, recombinant versions of these ASP antigens have been unsuccessful in eliciting a protective immune response. Prior research hypothesised on the importance of glycosylation in achieving a protective immune response with these antigens. Consequently, this introduction will explore the nature of glycans, their potential impact on the immune response, and the existing knowledge regarding the glycosylation of the Oo-ASP-1 and Co-dd-ASP vaccine antigens. In essence, the primary aim of this thesis was to examine the role of N-glycans in the

immunogenicity of *O. ostertagi* and *C. oncophora* ASPs as vaccine antigens and to use this information to steer the production of new recombinant vaccine antigens.

In Chapter 2, the focus was on vaccine development against *O. ostertagi*, where the objective was to determine the structural characteristics of Oo-ASP-1 that are essential for inducing a protective immune response. This involved a comparison of the protein folding and N-glycosylation between the protective native Oo-ASP-1 and a non-protective recombinant Oo-ASP-1 produced in *Pichia pastoris*. Both antigens displayed a highly comparable protein structure, but their N-glycan compositions were different. Following this, the aim was to address these disparities in N-glycosylation, mostly concerning core fucosylation, through recombinant expression of Oo-ASP-1 in glyco-engineered *Nicotiana benthamiana* plants. These recombinants were subsequently assessed for their immunostimulatory and protective potential as a vaccine antigen in a bovine immunisation-infection study. The immunisation of calves with these plant-produced recombinants led to a significant 39% reduction in parasite egg output, similar to the protective efficacy of the native antigen. These results underscore the importance of N-glycosylation in these vaccine antigens when it comes to eliciting a protective immune response. In addition, the procedural framework introduced in this chapter could be of significant importance in advancing the development of recombinant vaccines against other parasitic nematodes.

Chapter 3 focussed on *C. oncophora*, where previous experiments had highlighted the high level of protection provided by immunisation of calves with a double-domain activation-associated secreted protein (Co-dd-ASP) against this nematode. Despite these promising results, obtaining an effective recombinant vaccine has been challenging, with no success thus far. Given the limited documentation of native Co-dd-ASP's N-glycosylation, this chapter concentrated on unravelling the antigen's glycosylation pattern, revealing the presence of various N-glycan types, including oligomannose, hybrid, and complex structures. The importance of N-glycosylation in the immune response became evident through glycan microarray analyses, where calves immunised with native Co-dd-ASP developed a glycan-reactive IgG response. New recombinant versions, incorporating a limited selected subset of oligomannose-type N-glycans, were produced in *Nicotiana benthamiana* plants. However, in a competitive antibody-binding assay, these recombinants failed to effectively compete with the native Co-dd-ASP for antibody binding, suggesting the existence of structural disparities. These complications were associated with the stability of the double-domain protein structure and, potentially, the complexity of mimicking the native antigen's N-glycosylation profile.

Chapter 4 assessed the protective capacity of low and mid molecular weight (LMW and MMW) protein fractions of adult *C. oncophora* ES material, aiming to obtain an alternative antigen for vaccine development in addition to the previously mentioned Co-dd-ASP. Immunisation of calves with the MMW fraction induced protection against *C. oncophora*, demonstrated by a significant 83% reduction in cumulative faecal egg excretion compared to control animals. In the next stage, a single domain ASP antigen (Co-sd-ASP) was purified from this MMW fraction and used to immunise calves, resulting in a significant reduction of faecal egg output by 86% and adult worm burden by 63%. In light of these promising results, establishing Co-sd-ASP as a vaccine candidate, recombinant expression of this antigen was conducted in *Nicotiana benthamiana*. Most notably, via recombinant expression it was possible to closely replicate the N-glycosylation profile of native Co-sd-ASP. Therefore, this recombinant is a strong candidate for further examination of its capacity to confer protection in a bovine immunisation-infection study.

Chapter 5 is dedicated to a discussion on how the findings in this thesis contribute to a deeper understanding of the role of glycosylation in the immunogenicity of these ASP antigens. The first section delves into the potential for further assessing the impact on vaccine efficacy, considering the availability of well-defined glycovariants of ASP. It also explores which studies could provide additional insights into the influence of glycosylation on various facets of the immune system, including the role of ASP-related glycans on DC activation. The second section addresses future considerations for recombinant expression, including optimising the N-glycosylation profile via expression in *N. benthamiana* and evaluating the potential use of *P. pastoris* and mammalian cell lines for the production of recombinant Oo-ASP-1, Co-dd-ASP, and/or Co-sd-ASP. The following section explores some practical considerations for a vaccine against gastrointestinal nematode infections in cattle and outlines the essential steps necessary for the ongoing development of these vaccines. To conclude, it delves into how the findings presented in this thesis can be instrumental for the advancement of recombinant vaccines production against nematode infections in general.