SCIENCES & BIOENGINEERING SCIENCES

The Research Group

Structural Biology Brussels (SBB)

has the honor to invite you to the public defense of the PhD thesis of

Charlotte VANMARSENILLE

to obtain the degree of Doctor of Bioengineering Sciences

Joint PhD with Ghent University

Title of the PhD thesis:

Protecting chickens against *Campylobacter jejuni* by *Camelidae*-derived nanobodies

Promotors:

Prof. Dr. Henri De Greve
Prof. Dr. Frank Pasmans (Ghent University)

The defence will take place on

Monday March 26th 2018 at 17:00h

in Auditorium D.2.01 at the Campus Humanities, Sciences and Engineering of the Vrije Universiteit Brussel, Pleinlaan 2 - 1050 Elsene, and will be followed by a reception.

Members of the jury:

Prof. Dr. Daniël Charlier (chairman)
Prof. Dr. Niek Sanders (secretary, Ghent University)
Prof. Dr. Jean-Pierre Hernalsteens (co-promoter)
Prof. Dr. An Martel (co-promoter, Ghent University)
Prof. Dr. ir. Geert Angenon
Prof. Dr. Peter Geldhof (Ghent University)
Prof. Dr. Delphine Martiny (CHUB-ULB)
Dr. Geertrui Rasschaert (ILVO)

Curriculum vitae

Charlotte Vanmarsenille obtained her Master in Bioengineering Sciences at the VUB. Subsequently she started a joint PhD in the research groups of Structural Biology Brussels and of Viral Genetics at the VUB and in the Department of Pathology, Bacteriology and Avian Diseases at Ghent University. Her research resulted in a first author publication in a peer reviewed journal and she presented her research at national and international conferences in poster presentations. She has supervised eight master theses and was involved in the laboratory course Microbiology and Gene Technology. The project was funded by the Federal Public Service Health, Food Chain Safety and Environment.

Abstract of the PhD research

Worldwide, *Campylobacter* is the most prevalent cause of foodborne infections in humans. Observed symptoms range from diarrhoea to neurological disorders and have a severe health burden as result. *C. jejuni* and *C. coli* are the two most common zoonotic species involved in human infections. Broiler chickens are the primary source for transmission. Infections are mostly due to consumption or handling of contaminated poultry meat. Control strategies that impede the colonization of poultry by *Campylobacter,* can be effective in reducing *Campylobacter*-related infections in humans. Despite major health issues and high societal costs associated with campylobacteriosis, efficient mitigation is not available and therefore novel control measures are required. In this context, we developed a passive immunization strategy, using *Campylobacter*-specific nanobodies, that has the potential to control colonization of chickens.

Initially, we isolated nanobodies directed against *Campylobacter,* using the phage display technology. Broad specificity of the nanobodies is interesting for wide protection, as broilers can be colonized by multiple *Campylobacter* strains. The selected broad specific nanobodies were shown to bind with surface-exposed proteins of *C. jejuni* and *C. coli* strains isolated from poultry and human patients. Nanobodies targeting the flagella and the major outer membrane protein (MOMP) were identified. Both are *C. jejuni* colonization-associated factors, involved in virulence. Flagella are required for motility and the MOMP is essential for viability and contributes to adhesion to host cells. The latter, the immunodominant character and the fact that MOMP is abundantly present in all *Campylobacter* strains, make the MOMP an interesting target for vaccine development. Nanobodies multimerized on magnetic beads agglutinate *C. jejuni* cells, which demonstrates that multimeric nanobodies can possibly promote clearance of the bacteria *in vivo*. Different methods for the multimerization of the nanobodies were evaluated. The most successful, was the fusion of two nanobodies to the effector domains of chicken immunoglobulins. Hereby, the benefits of a nanobody are combined with a bivalent character and an increased half-life, which is interesting for therapeutic purposes. The chimeric anti-*Campylobacter* antibodies were expressed in *Arabidopsis thaliana* seeds and in the yeast *Pichia pastoris*. Both expression platforms were used for the production of chimeric antibodies interacting with *Campylobacter* bacteria. The work described in this study, forms the basis for the development of a passive immunization approach for the protection of chickens against *Campylobacter* colonization.