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INVITATION
PUBLIC PhD DEFENSE

Prevalence and virulence potential of 'gang of five' Shiga toxin-producing *Escherichia coli* (STEC) on dairy cattle farms and assessment of heterologous vaccination to reduce faecal excretion of STEC O26:H11 in calves

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PROMOTORS

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Curriculum Vitae

Frederik Engelen was born on December 5th, 1992 in Turnhout, Belgium. In 2016, he obtained his master's degree in Pharmaceutical Sciences (Minor Drug Development) from the Catholic University of Leuven (KUL), Belgium. Following his graduation, he started his doctoral studies in December 2016 at the Laboratory of Immunology, Faculty of Veterinary Medicine, Ghent University (UGent), Belgium. His work focused on the occurrence of Shiga toxin-producing *Escherichia coli* (STEC) on Belgian dairy cattle farms and evaluation of vaccination as a potential strategy to reduce bovine STEC excretion in calves. Frederik is author of several publications in peer-reviewed international journals, of which two he is first author. He participated in several (inter-) national scientific conferences and obtained the Doctoral Training Programme certificate of the Ghent University Doctoral Schools.

How to attend?

The public defense will take place on Monday, May 3, 2021 at 2:00 PM.
Due to the COVID-19 restrictions, the public defense will be virtual.
If you would like to attend, please contact Frederik Engelen before April 26th via frederik.engelen@ugent.be

Members of the Examination Committee

Prof. dr. G. Opsomer, Chairman, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

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Summary

Shiga toxin-producing *E. coli* (STEC) are important zoonotic foodborne pathogens causing acute and bloody diarrhoea, potentially progressing to the life-threatening haemolytic-uremic syndrome (HUS). Cattle represent the major natural reservoir and asymptomatically carry STEC in their gastro-intestinal tract, resulting in faecal excretion of these pathogens. Consequently, human infection occurs predominantly through ingestion of food and/or water contaminated with bovine faeces. STEC are characterized by their ability to produce Shiga toxins 1 and/or 2 (Stx1, Stx2). In addition, most STEC strains associated with severe human disease also possess the locus of enterocyte effacement (LEE) pathogenicity island, encoding the outer membrane adhesin intimin (*eae*), its translocated intimin receptor (Tir), a type three secretion system (T3SS) and several translocated effector molecules, responsible for the formation of characteristic attaching-effacing (A/E) lesions which mediate intimate attachment of STEC to the intestinal mucosa. Over 400 STEC serotypes have been identified to date, but only a limited number have been associated with human pathogenicity. While STEC O157:H7 is the serotype most frequently associated with severe human illness, several non-O157 serotypes have emerged over the last decades as important pathogens. Since effective treatments for STEC infections in humans are currently non-existent, the development of interventions aimed at reducing the occurrence of STEC in its bovine reservoir has received considerable attention over the last decades.

Chapter 1 summarizes the current knowledge on both STEC O157:H7 and non-O157 and discusses important insights into their pathogenesis, animal reservoir, epidemiology and evoked immune response. Additionally, intervention strategies aimed at reducing bovine STEC excretion are reviewed, with a special focus on cattle vaccination.

The general aim of this study (**Chapter 2**) was to gain insight in the occurrence and pathogenic potential of 'gang of five' STEC serogroups on Belgian dairy cattle farms, both at farm level and animal level, and to investigate cross-protection against STEC O26:H11 infection by vaccination of calves with STEC O157:H7 antigens.

In our first field study (**Chapter 3**), the occurrence and pathogenic potential of 'gang of five' STEC serogroups was investigated on Belgian dairy cattle farms. These so-called top-five STEC serogroups, being O157, O26, O103, O111 and O145, consistently sit in the first 10 ranks of STEC serogroups in human infections reported in the EU in the last 5 years. For this reason, they were given a special spotlight in this study. Nineteen Belgian dairy cattle farms were screened for presence of 'gang of five' STEC serogroups by overshoe (OVS) sampling of the pen beddings. Overall, 11 out of 19 farms (58%) tested positive for 'gang of five' STEC, with O26 STEC most frequently isolated from OVS (11/88; 12.5%), followed by O157 (10/88; 11.5%), O145 (3/88; 3.5%) and O103 (3/88; 3.5%). The highest proportion (35%) of 'gang of five' STEC positive OVS was found in young cattle between 1-24 months of age, as compared to new-born calves (11%) and adult cattle (21%). Importantly, half of the obtained 'gang of five' STEC isolates (48%) possessed both the *eae* and *stx2* gene, suggesting a high pathogenic potential to humans. Overall, these results demonstrated that (i) OVS sampling can serve as a practical first-line screening method to determine the 'gang of five' STEC status of cattle farms, (ii) young calves seem to be more at risk of harbouring potentially pathogenic STEC as compared to adult cattle, and (iii) O157 and O26 are the two most prevalent 'gang of five' STEC serogroups circulating on dairy cattle farms.

Consequently, these findings motivated us to further investigate the occurrence and pathogenic potential of the two most prevalent STEC serogroups, namely O157 and O26, in young dairy calves (**Chapter 4**). Recto-anal mucosal swabs (RAMS) were collected from healthy and diarrhoeic <6-month-old dairy calves on three dairy cattle farms that previously tested positive for both STEC O157 and STEC O26 by OVS sampling. Overall, 19% of RAMS tested positive for *E. coli* O157, while 31% tested positive for *E. coli* O26. In addition, the majority of isolates possessed both *eae* and *stx*, denoting a high pathogenic potential to humans. While both *E. coli* serogroups persisted at farm level, persistence within the same animal over time appeared to be relatively rare, with only 5 out of 66 animals (8%) testing positive for the same serogroup on multiple sampling points. Interestingly, *E. coli* O26 was already abundantly present at a younger age compared to *E. coli* O157. Furthermore, calf diarrhoea could not be associated with presence of *E. coli* O26.

Since STEC O157:H7 and O26:H11 are responsible for the majority of human STEC infections, a bovine vaccine capable of reducing the faecal excretion of these two serotypes in cattle would be highly beneficial from a public health point of view. Therefore, **Chapter 5** focused on the effect of vaccination of calves with STEC O157:H7 antigens on the faecal excretion of STEC O26:H11. While vaccination induced clear serum antibody responses and resulted in a significant reduction in faecal excretion of STEC O26:H11 in previously infected calves, it failed however to protect against re-infection with STEC O26:H11.

The final part of this thesis (**Chapter 6**) provides the general discussion, main conclusions and future perspectives of this work. Overall, the work described in this thesis demonstrates not only that young dairy calves are important reservoirs of potentially pathogenic *E. coli*, but also that vaccination of calves has the potential to significantly reduce faecal excretion of these pathogens, thus contributing to a reduction in human infections. The results presented here can be used to guide future studies on STEC prevalence in cattle, as well as to inform the future development of bovine STEC vaccines.