Promoter

Prof. Dr. Hans. J. Nauwynck

Faculty of Veterinary Medicine, UGent

Members of the Examination Committee

Prof. Dr. Piet Deprez

Faculty of Veterinary Medicine, UGent

Chairperson of the Examination Committee

Prof. Dr. Herman Favoreel

Faculty of Veterinary Medicine, UGent

Prof. Dr. Richard Ducatelle

Faculty of Veterinary Medicine, UGent

Prof. Dr. Benjamin G. Dewals

Faculty of Veterinary Medicine, University of Liege

Dr. Lennert Steukers

Janssen Pharmaceutica NV, R&D Therapeutic Areas, Beerse

Dr. Bert Devriendt

Faculty of Veterinary Medicine, UGent

Curriculum Vitae

Jing Zhao was born in 1987, in Binzhou City, Shandong Province, China.

In June 2009, she obtained her Bachelor degree in Veterinary medicine from Liaocheng University in China and graduated with the honor of Excellent Graduates of Shandong Province in June 2009. In the same year, she was enrolled as a master student in Harbin Veterinary Research Institute, Chinese Academy of Agricultural Sciences, studying the preparation of monoclonal antibodies against E2 protein of Eastern equine encephalitis virus and identification of B-cell epitopes in National Key Laboratory of Veterinary Biotechnology. And she attended the training course entitled "International Research on Epidemiology of Zoonoses and Training for Young Researchers" at the Veterinary Medical Research Center at Nihon University in Japan in February 2010. In June 2012, she obtained a Master degree in Prevention Veterinary Medicine.

In September 2012, she started her PhD training under supervision of Prof. Hans Nauwynck in the Laboratory of Virology, Department of Virology, Parasitology and Immunology, Faculty of Veterinary Medicine, Ghent University on the project "*Invasion of equine and bovine alphaherpesviruses through the respiratory mucosa and interaction between equine herpes- and arteritis virus"*. Her PhD project was sponsored by the Chinese Scholarship Council (CSC), the Concerted Research Action 01G01311 of the Research Council of Ghent University, and Belgian Science Policy (BELSPO). She is the author and co-author of several publications in international peer-reviewed journals.

200 YEARS
GHENT UNIVERSITY

INVITATION

Public Defence of the Doctoral Thesis of

Jing Zhao

May 12th, 2017

Laboratory of Virology
Department of Virology, Parasitology
and Immunology
Faculty of Veterinary Medicine, UGent

FACULTY OF VETERINARY MEDICINE
accredited by EAEVE

You are kindly invited to attend the public defence of the doctoral thesis of

Jing Zhao

Title of the thesis:

Invasion of equine and bovine alphaherpesviruses through the respiratory mucosa

The public defence will take place on
Friday, May 12th, 2017
at 17:00 hours
Auditorium Hoogbouw
Faculty of Veterinary Medicine
Salisburylaan 133, 9820 Merelbeke

After the public defence a reception will be held in the Museum of Anatomy

---

Please confirm your attendance before May 3rd, 2017 to
Jing.Zhao@Ugent.be

Summary of the thesis

Bovine herpesvirus 1 (BHV-1) is able to induce prominent epithelial plaques and penetrate the basement membrane (BM) in a plaque-wise manner in the respiratory mucosa. The directional transport of BHV-1 towards the BM is important for virus dissemination and invasion to penetrate the BM. BHV-1 Us3 can alter intracellular localization of the virus and has been found to colocalize with microtubules in cell protrusions, which implicates possible direct interactions of Us3 with kinesin or dynein motors. Us9 together with kinesins might also contribute to the directional transport of alphaherpes viral glycoproteins in the epithelial cells of the respiratory mucosa. Thus, we examined the function of Us3 and Us9 protein on the stromal invasion of BHV-1 in the respiratory mucosa.

Equine herpesvirus 1 (EHV-1) and equine arteritis virus (EAV) are two important pathogens of horses, which have significant economical impacts on the equine breeding industry worldwide every year. In the upper respiratory mucosa, $CD172a^+$ monocytic cells become infected with both EHV-1 and EAV and transport the viruses to the lymph and blood circulation for further invasion. Up to now, commercial vaccines do not provide full protection against EHV-1 infection and there is no efficacious antiviral treatment available. As $CD172a^+$ monocytic cells function as Trojan horses in the respiratory mucosa, inhibition of the recruitment of these monocytic cells may prevent migration of infected monocytic cells into the deep tissues. Thus, we examined whether $CD172a^+$ monocytic cells are specifically recruited to the EHV-1 infection sites in order to capture virus and whether treatment with dendritic cell migration inhibitors rosiglitazone or quinacrine in the respiratory mucosa can impede EHV-1 deeper invasion. In addition, as the clinical outcomes of EHV-1 and EAV infections are indistinguishable under field conditions, a study on the behavior of dual infections with EHV-1 and EAV in the equine respiratory mucosa was performed.

In **Chapter 2**, the aims of this thesis were formulated.

In **Chapter 3,** it was found that Us3 and Us9 play an important role in the invasion of BHV-1 through the BM of the respiratory mucosa. Knockout of Us3 resulted in a remarkable reduction in viral titer and plaque size (latitude) in MDBK cells and trachea mucosa while there was no defect on cell-to-cell spread observed for BHV-1 Us9 null virus. Both BHV-1 Us3 null and Us9 null viruses showed a significant reduction of plaque penetration underneath the BM, however the penetration was not completely inhibited.

In **Chapter 4,** it was shown that with neurovirulent strains but not with the non-neurovirulent strains, $CD172a^+$ monocytic cells specifically migrated towards EHV-1 infected regions and that CCL2 and CCL5 were involved. CCL2 started to be expressed in infected epithelial cells at 24hpi and CCL5 at 48hpi, which corresponded with the $CD172a^+$ monocytic cell migration. Rosiglitazone but not quinacrine decreased the migration of $CD172a^+$ monocytic cells in the lamina propria without having an effect on the virus replication in the epithelium.

In **Chapter 5,** it was found that an inhibitory effect induced by either EHV-1 or EAV in the dual infections was present in RK-13 cells regardless of the order of virus exposure. In addition, RK-13 cells co-infected with both EHV-1 and EAV were not found upon superinfections. Only a few were found in co-infections. In nasal and nasopharyngeal mucosae, there was no effect of an EAV infection on EHV-1 replication in the respiratory epithelium. EAV and EHV-1 pre-infections slightly reduced the number of EHV-1 and EAV infected leukocytes compared to the single infections and co-infection. In double EAV and EHV-1 infected explants, no co-infected leukocytes were detected.

In **Chapter 6,** all data on invasion of equine and bovine alphaherpesviruses through the respiratory mucosa obtained in the present thesis were reviewed and discussed.

The main conclusions drawn in this thesis are:

BHV-1 Us3 and Us9 proteins play a crucial role in viral passage across the BM barrier during infection of bovine trachea mucosa explants. BHV-1 Us3 protein also contributes to the lateral cell-to-cell spread in both MDBK cell cultures and tracheal mucosa epithelium while BHV-1 Us9 null virus showed no defect.

A basal to apical migration of $CD172a^+$ monocytic cells was present in nasal mucosa during infections with neurovirulent EHV-1 strains, with CCL2 and CCL5 involved in the attraction of $CD172a^+$ monocytic cells towards the infected regions. Rosiglitazone treatment efficiently inhibited the $CD172a^+$ monocytic cells migration in a dose-dependent manner while it had no effect on the virus replication in the epithelium of nasal mucosa.

There was a minor inhibitory effect of dual infections with EHV-1 and EAV *in vitro* and *ex vivo*, which is only limited during the early stage of infection. EHV-1 and EAV do not infect the same mucosal leukocytes.