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Curriculum Vitae

Haileleul Negussie Dubale was born in Ethiopia. In August 2002, he received a degree of Doctor of Veterinary Medicine (DVM) from the Faculty of Veterinary Medicine at the Addis Ababa University, Ethiopia. In October 2002, he started his professional career as a lecturer in the Department of Animal Health, Alage Technical and Vocational Education and Training College. In September 2007, he continued his master studies in the Faculty of Veterinary Medicine at the Addis Ababa University, Ethiopia. He did his master thesis with a project on "molecular epidemiology and vaccine matching study on foot and mouth disease virus isolated in Ethiopia". In June 2009, he obtained a Master's degree in Veterinary Epidemiology. Haileleul joined the Faculty of Veterinary Medicine at Addis Ababa University, Ethiopia in 2009 as an Assistant Professor of epidemiology and preventive medicine. In October 2012, he started a PhD program under the supervision of Professor Hans Nauwynck in the Laboratory of Virology, Faculty of Veterinary Medicine at Ghent University. During his PhD studies, he worked on equine herpesviruses. His PhD program was funded by "The Special Research Fund (BOF)" of the Ghent University. He is author and co-author of several publications in national and international peer-reviewed journals.

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INVITATION

Public Defense of the Doctoral Thesis of

Haileleul Negussie Dubale

January 16, 2017

Laboratory of Virology
Department of Virology, Parasitology and Immunology
Faculty of Veterinary Medicine
Ghent University

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

Haileleul Negussie Dubale

Title of the thesis:

Epidemiology of equine herpesviruses (EHVs) in Ethiopian equids and invasion characteristics of EHV-1 and EHV-3 in respiratory and genital mucosae

The public defense will take place on
Monday, January 16, 2017
at 17:30 hours
Auditorium Hoogbouw
Faculty of Veterinary Medicine
Salisburylaan 133, 9820 Merelbeke

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

Summary of the thesis

The equine herpesviruses (EHVs) are ubiquitous pathogens in equine populations worldwide. They establish a lifelong latent infection, which ensures the survival of herpesviruses in equine populations. The currently available modified live and inactivated products of EHV-1 and EHV-4 vaccines are not reliably providing protection against infection. A better understanding of the epidemiology of EHV infections in natural outbreaks is particularly important to formulate an effective intervention strategy. However, many data gaps exist and more investigation needs to be done. It is very well known that the respiratory mucosal epithelium is the primary site of EHV-1 replication. However, EHV-1 infection in the vaginal mucosa is largely unknown. Moreover, information regarding the pathogenesis of EHV-3 in the nasal and vaginal mucosa is limited.

In Chapter 1, an overview of the current knowledge of EHVs and the characteristics of the respiratory and genital mucosae were given. In this chapter, an introduction was given on the historical background, the taxonomy, the general herpesvirion structure, the replication cycle, the epidemiology, the pathogenesis and the EHV-associated clinical signs. We also provided the general characteristics of upper respiratory and genital tract mucosal tissues with residing immune cells.

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

In Chapter 3.1, the epidemiology and molecular characteristics of EHV-1 from clinically EHM-affected Ethiopian equids during outbreaks from May 2011 to December 2013 were described. We recorded a higher incidence of EHM outbreaks from April to mid-June. There were no visible differences in the clinical presentation of EHM among horses, mules, and donkeys. However, EHM in donkeys was more severe and death after showing clinical signs of ataxia and paresis. EHM affected equids mainly recorded over three years of age and the age ranged from 7 to 10 years were largely affected. Further, we also documented that females were more affected than males. The incidence of neuropathogenic (D_{752}) and non-neuropathogenic (N_{752}) variants of EHV-1 from EHM-affected Ethiopian equids was assessed by sequencing the DNA polymerase gene (ORF30) of the EHV-1 isolates. The results indicated that 98.9% (90 out of 91) equids were infected with an ORF30 D_{752} variant. Analysis of ORF68 as grouping marker for geographical differences showed that the Ethiopian EHV-1 isolates belonged to geographical group 4.

In Chapter 3.2, we described the detection and genetic characterization of EHVs from equids with and without clinical respiratory disease. Virus-specific PCRs were used to detect EHV-1, -2, -4, and -5. From the total of 160 equids with respiratory disease, EHV-5 was detected at the highest prevalence (23.1%), followed by EHV-2 (20.0%), EHV-4 (8.1%), and EHV-1 (7.5%). Concurrent infections with EHV-2 and EHV-5 were recorded from 9 (5.2%) diseased horses. Of the total of 111 clinically healthy equids, EHV-1 and EHV-4 were never detected whereas EHV-2 and EHV-5 were found in 8 (7.2%) and 18 (16.2%) horses, respectively. A significantly higher proportion of EHV-2-infected equids was observed in the respiratory disease group (32/160, 20.0%; P = 0.005) compared to those without disease (8/111; 7.2%). EHV-2-positive equids were three times more likely to display clinical signs of respiratory disease than EHV-2-negative equids (OR 3.22, 95% CI: 1.42 to 7.28). For EHV-5, the observed difference was not significantly (P = 0.166) different. The phylogenetic analysis of the gB gene revealed that the Ethiopian EHV-2 and EHV-5 strains had a remarkable genetic diversity. Our results suggest that besides EHV-1 and EHV-4, EHV-2 is likely to be an important contributor either to induce or predispose equids to respiratory disease.

In Chapter 4, the replication kinetics and invasion characteristics of EHV-1 and EHV-3 in nasal and vaginal mucosae were compared using explants. The explants were cultured during 96 h with little change in viability. The tissues were inoculated with EHV-1 03P37 (neuropathogenic), 97P70 (abortigenic), and EHV-3 04P57, collected at 0, 24, 48, and 72h pi, and stained for viral antigens. The plaques were already observed at 24 h pi, their size increased over time and did not directly cross the basement membrane. However, EHV-1 infected the monocytic cells and hijacked these cells to invade the lamina propria. In contrast, EHV-3 replication was fully restricted to epithelial cells; the virus did not breach the basement membrane via a direct cell-to-cell spread nor used infected monocytic cells. EHV-1-induced plaques were larger in the nasal mucosa compared to the vaginal mucosa. The opposite was found for EHV-3-induced plaques. Both EHV-1 strains replicated with comparable kinetics in the nasal mucosa. However, the extent of replication of the abortigenic strain in vaginal mucosa was significantly higher than that of the neuropathogenic strain. Two-to-five-fold lower numbers of EHV-1-infected monocytic cells underneath the BM were found in the vaginal mucosa than in the nasal mucosa. Our study has shown that (i) EHV-1 has developed in evolution a predisposition for respiratory mucosa and EHV-3 for vaginal mucosa, (ii) abortigenic EHV-1 replicates better in vaginal mucosa than neuropathogenic EHV-1 and (iii) EHV-3 demonstrated a strict epithelial tropism whereas EHV-1 in addition hijacked monocytic cells to invade the lamina propria.

In **Chapter 5**, all data obtained in the present thesis were reviewed and discussed. A general conclusion on the research data generated in this thesis was formulated, in which the epidemiology of EHVs in Ethiopian equids and the new insights in the invasion behavior of EHV-1 and EHV-3 in the respiratory and vaginal mucosae were summarized.