FACULTY OF VETERINARY MEDICINE
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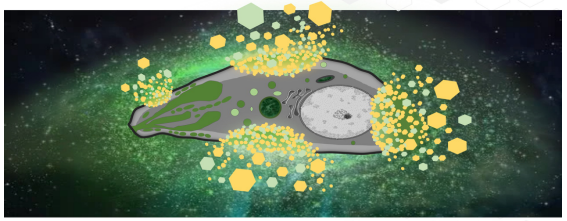
Invitation to the public defense of

the doctoral thesis

Toxoplasma gondii infection kinetics and immune responses following infection and vaccination



Md. Mizanur Rahman

25th February, 2020

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Curriculum Vitae

Md. Mizanur Rahman was born on 12th June, 1979 in Bogra, Bangladesh. In 2004, he obtained an B. Pharm in Pharmacy degree from the University of Rajshahi, Bangladesh, where he worked for six years as a Senior Executive, Product Management Department in Navana Pharmaceuticals Ltd., Bangladesh.

For the upgradation of his career, in academic year 2012-2014, he followed the Interuniversity program of Molecular Biology (IPMB), a master program sponsored by VLIR-UOS of Belgium and organized by the Free University of Brussels (VUB), the Katholieke Universiteit Leuven and the University of Antwerp (UAntwerp). During his master training, Mizanur did his thesis in the Lab of Immunology, Faculty of Veterinary Medicine, University of Ghent where he was inspired by the Toxosafe project, funded by Federal Public Service for Health, Food Chain Safety and Environment (grants RF 09/6213). He continued his 3.5 years PhD study on this project, focusing on *Toxoplasma gondii* infection kinetics and immune responses following infection and vaccination in pigs. Mizanur Rahman is the author and coauthor of several publications in peer-reviewed international journals and participated in several international conferences with oral or poster presentations.

The date and the venue:

You are cordially invited to the public defense that will take place on

Tuesday, 25th February 2020, 17:00.

Venue: Auditorium Hoogbouw (Ingang 24, 3rd floor, auditorium 1) of the Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, Merelbeke, Belgium.

After the defense you are kindly invited for the reception in the Museum of Morphology.

Please confirm your attendance before 20th of February, 2020 by phone (+32 466406279) or mail (Mizanur.Rahman@UGent.be).

Summary:

Toxoplasma gondii is an obligate intracellular parasite which often causes toxoplasmosis in humans and animals. It is estimated that about one third of the world population has undergone a *Toxoplasma* infection. Although most *T. gondii* infections are asymptomatic, it is a significant cause of fetal and neonatal mortality. *T. gondii* are highly infectious and most infections in humans are due to the consumption of raw or undercooked meat products contaminated with tissue cysts. *T. gondii* contaminated pork was estimated to account for 12-15% of human toxoplasmosis. However, there is a huge knowledge gap on the host immune response and the intervention strategies. As such, to generate sufficient data to bridge some of the knowledge gaps, this PhD thesis focused on *T. gondii* infection kinetics and immune responses and evaluated vaccine candidates in pigs.

Previous research by the lab of immunology showed that pigs infected with the *T. gondii* LR strain, followed by an infection with the *T. gondii* Gangji strain, resulted in a decrease in the parasite load in tissues. We hypothesized that intestinal immune responses shortly after infection might play a role in this strain-specific clearance. To assess this, we studied in chapter III the difference in both strains during the acute phase of infection in more detail, namely the parasite load in small intestinal lymph node cells and peripheral blood mononuclear cells (PBMC's) as well as the IFNγ secretion by these cells. Pigs were orally inoculated with tissue cysts of *T. gondii* LR or Gangji strains. Four days post inoculation with the LR strain, the parasite was detected by qPCR only in the duodenal mesenteric lymph node cells, while in other mesenteric lymph node cells and PBMC's the parasite was detected from day 8 post inoculation onwards. Although we observed a similar profile upon inoculation with the Gangji strain, the parasite load in the examined cells was much lower. This was reflected in a significantly higher *T. gondii*-specific serum IgG response in LR compared to Gangji infected pigs at day 28 post inoculation. Unexpectedly, this was not reflected in the IFNγ secretion upon re-stimulation of the cells with *T. gondii* total lysate antigen (TLA), which induced an almost equal IFNγ response in both groups. This difference between humoral and cellular immune response needs further studies.

Infection in pigs most often does not occur via uptake of tissue cysts, but by ingestion of oocysts via contaminated feed or water. It was therefore interesting to compare the parasite burden in edible tissues and the associated host immune responses in pigs upon infection with tissues cysts with this following infection with oocysts (Chapter IV). Upon oral inoculation with tissue cysts, TLA-specific serum IgG rapidly appeared in the acute phase of infection, whereas inoculation with oocysts showed a slower IgG response, reaching higher levels during the later phase of the infection. The kinetics of the IFNγ response upon *in vitro* re-stimulation of PBMCs was different for pigs infected with tissue cysts with a significantly higher secretion in the LR group at the first peak and in the Gangji at the second peak (*P=0.025*). However, oocyst inoculated pigs, only showed the early IFNγ peak. This lower IFNγ production might have contributed to the significantly higher *T. gondii* DNA load in the tissues of all oocyst inoculated pigs in comparison with tissue cyst inoculated pigs.

Vaccination remains the strategy of choice to reduce infection of farm animals, such as pig and sheep. Previous experiments suggested that the early immune response against *T. gondii* was important to reduce parasite load in tissues. The aim of Chapter V was to evaluate two potential vaccine candidates using an immunisation protocol in which the second immunisation was given shortly after oral inoculation of the animals with tissue cysts of *T. gondii* LR to ensure a strong immune response during the early phase of infection. The vaccine candidates were *T. gondii* lysate antigens (TLA) and a mixture of two fractions of TLA, generated by size fractionation, which induced a strong IFNγ response in an antigen recall assay. Our results showed that both vaccine candidates elicited strong serum IgG responses and elevated percentages of $CD4^+CD8^+IFN\gamma^+$ T cells in *T. gondii* infected pigs. However, the TLA vaccine induced the strongest immune response and reduced the parasite DNA load below the detection limit in brain and skeletal muscle tissue in most animals. These findings might inform the development of novel vaccines to prevent *T. gondii* infections in livestock species and humans.

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