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Serological Markers for Improved Diagnosis of Porcine Cysticercosis

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Taenia solium taeniosis/cysticercosis is an important (re)emerging zoonotic helminth infection in developing countries. Man is the definitive host, harboring the adult tapeworm in the small intestine. The natural intermediate host is the pig, but humans can also become infected with the larval stage, the cysticerci. In humans, cysticerci tend to lodge in the central nervous system, causing neurocysticercosis, which is a major cause of epilepsy in endemic countries.
Different serological tests are available for diagnosis of cysticercosis both in pigs and humans. Measuring antibody or antigen responses provides different information on the course of infection. In general, antibody detection assays only reflect exposure to the parasite whereas antigen detection assays indicate the presence of living parasites. The **first objective** of this thesis was to link serological data (antibody and antigen responses) to parasitological findings in experimentally infected pigs in order to study the host-parasite relationship.
The current monoclonal antibody based antigen-ELISA detects circulating parasite antigens, which indicate the presence of living cysts. However, no information is available on whether this test has potential for quantitating the number of viable cysts present or whether it is merely a qualitative measurement. Hence, the **second objective** was to study the relationship between number of cysts and titer of circulating antigen in pigs in order to develop a quantitative diagnostic test.
Diagnostic tests for porcine cysticercosis were recently validated based on a Bayesian approach. In a recent report by FAO, WHO and OIE, the development of more sensitive and specific diagnostic tests for use in pigs was stated as one of the research priorities for taeniosis/cysticercosis. For one, there is no test available that can distinguish between infections with viable cysts (active cysticercosis) and infections with degenerated cysts (inactive cysticercosis). Therefore, the **third objective** was to identify novel biomarkers in serum of infected pigs that can distinguish between active and inactive cysticercosis, using surface enhanced laser desorption and ionization time-of-flight mass spectrometry (SELDI-TOF MS).
Another gap in the diagnosis of porcine cysticercosis is the fact that the use of the antigen-ELISA in pigs is hampered by cross-reactions with other taeniid species. Hence, the **last objective** was to produce Nanobodies (camelid derived single domain antibody fragments) specific for *T. solium* that can be used for diagnosis of porcine cysticercosis.

In the first study, antibody and antigen levels were determined in experimentally infected pigs and linked to the parasitological outcome of infection. In the experimental model, 3 groups of pigs aged respectively 1, 3 and 5 months at infection were infected with a full proglottid. The 1-month old animals developed mainly viable cysts, the 5-month old animals mainly degenerated cysts, whereas the 3-month old animals showed an intermediate profile. All animals harboring viable cysts at necropsy had high antigen levels, whereas animals harboring no cysts or only degenerated cysts, had low or nil antigen levels. Antigen levels increased more rapidly in 1-month old animals harboring viable cysts than in 5-month old animals harboring viable cysts. Also, the antigen levels reached were higher in the animals infected at 1 month of age compared with animals infected at 3 and 5 months of age. Serum antibody levels appeared to follow a reverse kinetics compared with circulating antigen. Antibody titers were highest in animals infected at 5 months of age. In the animals infected at 1 and 3 months of age, antibody levels increased slowly and remained substantially lower compared with the older age group. These results indicate the presence of an age-dependent immune response: an efficient antibody response in older animals prevents the establishment of fully developed viable cysts whereas in younger animals the immune system cannot adequately react to the infection resulting in the establishment of viable cysts (accompanied by high circulating antigen levels).
In the second part of this study, a quantitative ELISA for measuring the concentration of circulating antigen was constructed using a reference standard curve of serial dilutions of ES products of *T. saginata*. A significant correlation between the number of viable cysts and the concentration of circulating antigen was found. This result is promising in view of the development of an assay to quantify the progress of an active *T. solium* infection.

In the second study, serum samples from the same experimentally infected pigs were analyzed by SELDI-TOF MS to identify biomarkers that can distinguish between infections with viable cysts and infections with degenerated cysts. Thirty discriminating biomarkers were found: 13 specific for the viable phenotype, 9 specific for the degenerated phenotype and 8 specific for the infected phenotype (either viable or degenerated cysts). Five biomarkers were identified as clusterin, lecithin-cholesterol acyltransferase (LCAT), vitronectin, haptoglobin and apolipoprotein A-I.
An attempt was made to validate the biomarkers by analyzing serum samples from naturally infected pigs. Only 3 of the biomarkers were also significant in the field samples; however, the peak profiles were not consistent among the two sample sets. Thus, it was not possible to validate the biomarkers.

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In the last study, Nanobodies were cloned following immunization of 2 dromedaries with *T. solium* cyst fluid and 8 *T. solium* specific Nanobodies were selected after phage display and biopanning. Their binding characteristics and potential for the diagnosis of porcine cysticercosis were investigated.
The Nanobodies were highly specific for *T. solium* and no cross-reactions with *T. hydatigena*, *T. saginata*, *T. crassiceps* and *T. spiralis* were observed. Western blotting of *T. solium* cyst fluid and immunodetection with the Nanobodies was performed. After transfer to a PVDF membrane and N-terminal sequencing of the proteins in the bands corresponding to the bands recognized by the Nanobodies, the target antigen was identified as 14-kDa diagnostic glycoprotein (Ts14), belonging to the 8-kDa protein family. Immunodetection was also assessed of four synthetic peptides belonging to this protein family (Ts14, Ts18var, TsRS1 and TsRS2). The Nanobodies preferably reacted with Ts18var1, only 2 Nanobodies also reacted with Ts14 and TsRS2. The complementation epitope groups of Nanobodies were investigated in competition ELISA. This way, the Nanobodies could be categorized in four different epitope-binding groups. Antigen capturing was assessed by testing the Nanobodies in various combinations in sandwich ELISA. The Nanobodies were able to distinguish between *T. solium* and *T. hydatigena* cyst fluid, however, there was no clear distinction between serum samples from *T. solium* infected pigs, *T. hydatigena* infected pigs and negative pigs. Next, the Nanobodies were tested in inhibition ELISA for the detection of circulating antigen. One Nanobody (Nbsol52) was able to differentiate between the different serum samples.
These results indicate the high potential of the selected Nanobodies for species-specific diagnosis of *T. solium* cysticercosis, after further assay optimization and validation.

The results of the present thesis demonstrate the feasibility to identify serological markers that can lead to improved diagnosis of porcine cysticercosis. The availability of an improved antigen detection assay provides clear prospects for epidemiological and immunological studies, follow-up of intervention studies and clinical monitoring in humans. In epidemiological studies, it can provide accurate information on sites of active transmission and risk factor assessment. Follow-up of cyst longevity is possible in immunological studies monitoring of control programs. In clinical cysticercosis in humans, it is a valuable decision making tool to start anthelmintic treatment and for follow-up of treatment efficacy.
To further elucidate host-parasite interactions and identify alternative target antigens for diagnosis, more fundamental research should be conducted. Therefore, the immune response in *Taenia* infections should be investigated, as well as comparative mapping of the parasite's proteome.

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List of articles published from thesis results

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