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# Abstract of Thesis/Dissertation

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Title :

Serosurveillance and vaccine development as a strategy for control of *Toxoplasma* infection.

(トキソプラズマ感染に対する制御方法の開発に向けた血清学的調査とワクチン開発)

## Abstract

Toxoplasmosis is a cosmopolitan infection caused by the intracellular protozoan parasite *Toxoplasma gondii*. Almost all warm blooded animals, including human can be infected with *T. gondii*. The disease has no apparent clinical forms in most cases other than immunocompromized or pregnant vulnerable hosts. Control of toxoplasmosis is considered a worldwide challenge which predominantly relied on accurate diagnosis and effective vaccination or therapeutic method. Accordingly, this study proposed the development of an effective control strategy for toxoplasmosis via assessing the current situation of the occurrence of *T. gondii* infection in Egypt and developing novel potent vaccine candidates.

Accurate demonstration and surveillance with appropriate methods is a critical step to recognize the current status of *T. gondii* infection in a certain area which is required for the development of suitable control policies. In chapter 1, a successful detection system was used to screen the specific antibodies against *T. gondii* in multiple animal species and in different regions of Egypt. The latex agglutination test is a reference test for detection of *T. gondii* infection and TgGRA7 (a parasite antigen)-based enzyme linked immunosorbent assay (ELISA) is a widely used method for surveying of the infection in the field. In this study, only the samples with simultaneously positive results for both tests (latex agglutination and TgGRA7-based ELISA) were considered positive. The collected samples were obtained from

different regions of Egypt and from all economically important animal species to establish a comprehensive record for *T. gondii* infection in Egypt. Among 652 sera obtained from animals of different localities in Egypt, 174 (25.7%) were identified as positive for anti-*T. gondii* antibodies. The prevalence in different animal species was 38.7%, 28.7%, 23.6% and 22.6% in sheep (43/111), goats (27/94), cattle (71/301) and donkeys (33/146), respectively. These results indicated the considerable occurrence of *T. gondii* infection in Egypt and implied such infection as a national challenge. Additionally, an epidemiological study was conducted to identify the effect of animal species (sheep, goats, cattle and donkeys), locality (Qena, Sohag, Giza, Minoufiya, Kafr El Sheikh and Matrouh) and climate (hot/dry and temperate/humid) as risk factors for the infection. The study revealed that sheep among other investigated animals and Kafr El Sheikh from other surveyed localities displayed the highest prevalence rate. Another study was undertaken to identify the risk factors for *T. gondii* infection in cattle and revealed that no significant differences between all investigated variables (age, sex, location and breeding system).

In chapters 2 and 3, establishment of an effective control strategy based on the development of potent vaccine candidates was proposed. According to the navigation results and previous reports and studies, *T. gondii*-derived peroxiredoxins (Prxs) were targeted for such task. Several reports revealed the potency of Prxs as vaccine candidates for various parasitic pathogens and in different animal models. Moreover, numerous *T. gondii*-derived enzymes induced protective immune response against challenge infection. Three Prxs were already identified in *T. gondii*. The TgPrx1 and TgPrx2 were identified in the cytoplasm of the parasite while TgPrx3 is localized in the mitochondria; all of them were expressed in tachyzoite and bradyzoite stages. Peroxiredoxin-linked detoxification of reactive oxygen species is identified as an efficient antioxidant system in *T. gondii*, protecting it against the resultant oxidative stress during its intracellular life. The TgPrx1 and TgPrx3 were evaluated as immunomodulators and novel vaccine candidates. To accomplish the aforementioned goal, the recombinant TgPrx1 and TgPrx3 were successfully expressed in *E. coli* by glutathione *S*-transferase (GST)-fusion system and the recombinant GST was used as a control protein. The endotoxin was removed from all protein lots before subsequent use in different immunological assays either in vitro or in vivo. Regarding macrophage immunoassays, polymixin B as an efficient endotoxin neutralizing agent was additionally

added to all stimulants to confirm the genuine stimulation of immune cells with the recombinant proteins and to validate the tests. To confirm the biological activity of recombinant protein of TgPrx1 and TgPrx3, serum samples of chronically infected mice with *T. gondii* were tested by indirect ELISA. Both TgPrx1 and TgPrx3 showed variable ability to recognize specific antibody, although they were not comparable to the known highly antigenic TgGRA7. The role of TgPrx1 and TgPrx3 was identified with promoting the macrophage function which was evidenced in the production of proinflammatory cytokine IL-12p40 but not IL-10 which has anti-inflammatory properties. This result suggested the ability of TgPrx1 and TgPrx3 to induce the Th1-mediated immunity, which is crucial for parasite restriction and elimination. Consequently, both TgPrx1 and TgPrx3 might possess a protective potential against *T. gondii* infection if they were used as a vaccine.

For vaccination study, C57BL/6 mice were subcutaneously injected with 25 pmol endotoxin-free recombinant proteins for three times by 14 days as interval, and then challenged with a lethal dose of *T. gondii* PLK strain via intraperitoneal route. The development of specific antibodies against TgPrx1 or TgPrx3 for IgG1 and IgG2c subclasses was efficiently observed especially after the second boost immunization. Moreover, in the recalling assay using spleen cells of immunized mice, the stimulation of TgPrx1 or TgPrx3-sentitized cells with the relevant antigen revealed the highest response for splenocytes proliferation, and IFN-γ and IL-4 production. Accordingly, these results indicated antigen- specific humoral and cellular mediated immune responses for both TgPrx1 and TgPrx3. According to the aforementioned results, the protective effects of TgPrx1 and TgPrx3 against *T. gondii* infection were speculated. After challenge, mouse groups were observed twice daily to record the survival and monitored for 30 days. Both TgPrx1 and TgPrx3 immunized mice exhibited higher survival rate in relation to the control groups which was significant in case of TgPrx1 but not for TgPrx3 while it was apparently higher. Moreover, the brains of surviving mice were collected, and the DNA was extracted by phenol-chloroform extraction method for quantification of the parasite load. Lower parasite burden in the brain of TgPrx1 and TgPrx3 than PBS or GST-immunized mice were noticed. These findings indicated the induction of resistance in the TgPrx1 and TgPrx3-immunized mice and implied their successes as novel vaccine candidates.

In conclusion, this study concluded the high occurrence and evenly distribution of *T.*

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*gondii* antibodies in Egypt, and offered the TgPrx1 as a novel potential vaccine candidate against toxoplasmosis.