別記様式第５号（第４条，第７条，第９条関係）

学　位　論　文　要　旨

博士後期 課程　畜産衛生学　専攻
学籍番号　27604
氏　　名　Gao Yang　印

論文題目：Functional characterization of *Toxoplasma gondii* SAG1-related sequence 2 and evaluation of its potential in serodiagnosis (トキソプラズマの SRS2 分子の機能解析と血清診断抗原としての評価)

要旨

Toxoplasmosis as an important worldwide zoonosis caused by *T. gondii*, an obligate intracellular protozoan parasite belonging to the phylum Apicomplexa. It has a worldwide distribution and is highly prevalent in many areas of the world. Researchers estimate that a third of the world's human population is infected with *T. gondii*, up to 95% in local areas, and about 200,000 new cases of toxoplasmosis occur every year. *T. gondii* can infect a wide range of hosts and has a facultative heteroxenous life cycle enabling it to survive in multiple hosts. The intermediate hosts which include almost all warm blooded animals, can harbor infective tissue cysts. The Felidae family, such as domestic cats, are the definitive hosts that pass the infective oocysts in their feces. Human and animals can be infected by ingestion of contaminated water, vegetables and meat products that contain infectious oocysts or tissue cysts. There are no clear symptoms of toxoplasmosis in healthy adults. However, toxoplasmosis is life threatening in immunocompromised individuals (such as those with HIV infection or on immunosuppressive therapies or post transplantation). In pregnant women, infection with the parasite can cause vertical infection resulting in stillbirth, neonatal malformations or abortion.

Development of accurate diagnostic methods is crucial for proper management and control of *T. gondii* infection in humans and animals. A number of diagnostic methods

have been developed and widely practiced. They include histologic demonstration, isolation of the organism, amplification of specific nucleic acid sequences and serological tests. Serological diagnosis is commonly used to detect antibodies against *T. gondii*. A number of serological procedures are available for detection of *T. gondii* antibodies, including Sabin-Feldman test, direct agglutination test, indirect fluorescent antibody assay (IFA), indirect hemagglutination assay, latex agglutination test (LAT), and enzyme-linked immunosorbent assay (ELISA). LAT is considered as the "gold standard" for detecting *T. gondii* infection, but has low specificity and a high cost. ELISAs seem to be the most suitable for routine mass diagnosis, e.g. sero-epidemiological investigations.

The SAG1-related superfamily (SRS) is one of coccidian-specific multicopy gene families of *T. gondii*. A TgSRS gene usually contains one or two domains, with every domain consisting of four to six cysteines. Theses cysteines involve a disulfide bond and a glycophosphatidylinositol (GPI) anchor to attach to the cell surface. TgSRS proteins are the differential expression of numerous closely related GPI-anchored surface proteins. They are expressed in a unique developmentally modulated manner as different, largely non-overlapping sets of TgSRS antigens which are thought to dominate the surface of *T. gondii*. The TgSRS antigens might provide some types of protective barrier, act as diverse arrays of cellular receptors, and/or function as immune activators or mediators of immune evasion that facilitates parasite survival and propagation. SAG1-related sequence 2 (TgSRS2) is a major tachyzoite surface antigen and has been identified to be expressed on the surface of *Toxoplasma* tachyzoites. In a previous study, the survival rate of mice significantly increased when infected with lethal dose of TgSRS2 overexpressing mutant parasites. These indicate that TgSRS2 may be an important virulence factor in *T. gondii*.

The main purpose of this study was to develop an accurate diagnostic method against toxoplasmosis. An in-depth understanding of the antigens that play a key role in the

growth and virulence of parasites is important in developing a specific and sensitive diagnostic tool. The aims of the current study are as follows: (1) to preferably understand the function of TgSRS2 in *T. gondii*; (2) to develop a diagnostic tool utilizing rTgSRS2 and evaluate its performance using sera form infected mice and clinical cat samples; and (3) to determine the seroprevalence of *T. gondii* infection in sheep in northern China using the developed rTgSRS2-based ELISA.

In chapter 1, functional analysis of TgSRS2 in RH and PLK strains was performed using CRISPR/CAS9 technology. Amino acid sequence alignment showed that TgSRS2 contained four polymorphic amino acids. Invasion assay showed that the percentage of cells infected by ΔTgSRS2 PLK strain was significantly lower than that of those infected by the parental strain. In addition, deletion of TgSRS2 significantly decreased the virulence of the PLK strain in mice. However, no difference was observed between the phenotypes of ΔTgSRS2 RH and parent strains, which could be due to the low expression of TgSRS2 in RH strain. These results indicate that TgSRS2 plays an important role in invasion and virulence of *T. gondii* PLK strain in mice.

In chapter 2, the immunogenicity and antigenicity of rTgSRS2 was evaluated using serum from mice experimentally infected with *T. gondii* and clinical cat samples in an ELISA reaction. The performance of the rTgSRS2 based ELISA was compared with that of LAT. The specificity and sensitivity of the ELISA were 95% and 71%, respectively. ELISA showed high concordance (84%) and substantial agreement (kappa value > 0.61) with the results of LAT. These results suggest that the rTgSRS2-based ELISA could be a useful tool for routine testing in clinical and mass screening of samples in the field.

In chapter 3, a survey was performed to evaluate the seroprevalence of *T. gondii* infection in sheep from northern China and to assess the risk factors for infection using LAT and rTgSRS2-based ELISA. Of the 288 serum samples, 87 samples (30.2%), 101

samples (35.1%), and 73 samples (25.5%) were positive for anti-*T. gondii* antibodies according to LAT, ELISA, and both tests, respectively. Seroprevalence of *T. gondii* infection in sheep was 53.7% in Hebei, 38.8% in Heilongjiang, 29.2% in Jilin and 19.7% in Inner Mongolia using ELISA. Age and rearing system significantly affected seropositivity. The findings obtained in this study may be helpful in designing appropriate measures for the prevention and control of toxoplasmosis in sheep, other animals and humans.

Overall, the present study analyzed the function of SRS2 in *T. gondii*, and evaluated the sensitivity and specificity of rTgSRS2 based ELISA and determined the seroprevalence of *T. gondii* infection in sheep in northern China using this ELISA method. These results provide useful information for understanding the functional characteristic of TgSRS2 and its ability to detect *T. gondii* infection in cats and sheep.

備考　1　論文題目が英語の場合には，（　）書きで和訳を付す。

2　博士論文については，日本語の場合１８００～２２００字，英語の場合１０００～１４００語とする。修士論文については，それ以下でもかまわない。

3　図表は，要旨には記載しないこととする。

4　枚数は１枚を超えても差し支えない。