Form No.5

Abstract of Thesis/Dissertation

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Title : Seroepidemiological study of *Toxoplasma gondii* and *Neospora caninum* in livestock in Mongolia and characterization of antiprotozoal compounds from soil bacteria in Mongolia

(モンゴルの家畜におけるトキソプラズマ・ゴンディとネオスポラ・カニナムの血清疫学調査およびモンゴルの土壌細菌由来抗原虫化合物の解析)

Abstract

Toxoplasmosis and neosporosis affect the livestock industry by decreasing productivity of livestock. *Neospora caninum* can cause abortion storms and high culling rates in cattle, whereas *Toxoplasma gondii* infection is a significant concern in both human and animals because it can induce abortion and clinical symptoms in immunocompromised hosts. Those infections may be a hazard to the livestock industry in Mongolia as a main sector of the economy in the country. The total livestock population consists of cattle, sheep, goat, camel and horses and those animals are used for different purposes in the country. Among them, cattle, sheep and goats are the main sources of foods for Mongolians and meat products derived from those animals are served as foods for daily lives. Because of the limited information of *T. gondii* and *N. caninum* infections, my present study proposed to determine large-scale seroprevalence of *T. gondii* and *N. caninum* and identify major risk factors in livestock in Mongolia. In addition, antiprotozoal compounds from soil bacteria in Mongolia were characterized in

order to develop effective chemotherapeutic agents against toxoplasmosis and Malaria.

In chapter 1, a total of 1,438 cattle sera from 20 of 21 provinces of Mongolia and the capital city of Ulaanbaatar were tested. Specific antibodies to *T. gondii* and *N. caninum* were detected by using an indirect enzyme-linked immunosorbent assay (iELISA) based on recombinant antigens of dense granule protein 7 of *T. gondii* and surface antigen 1 of *N. caninum*, respectively. Overall, 18.7% and 26.2% of cattle were positive for specific antibodies to *T. gondii* and *N. caninum*, respectively. Prevalence rates were higher (*T. gondii* infection: $P < 0.0001$, *N. caninum* infection: $P = 0.002$) in the central region of Mongolia (*T. gondii* infection: 27.1%, *N. caninum* infection: 30.8 %) compared with western region, suggesting that prevalence rates might be influenced by geographical condition, particularly warmer temperatures around this area in Mongolia. The lowest prevalence rates were observed in the western region of Mongolia (*T. gondii*: 9%, *N. caninum*: 20.8%). In addition, the seroprevalence of *N. caninum* in female animals (27.5%) was significantly higher than that in male animals (20.4%) ($P = 0.018$), suggesting an important risk factor of abortion and stillbirth in cattle. I investigated the mixed infection with *T. gondii* and *N. caninum* in cattle in Mongolia. The overall seroprevalence rate was 6.8%, based on this data, the mixed infection with *T. gondii* and *N. caninum* are not commonly occurred in Mongolia. The present results showed that *T. gondii* and *N. caninum* infections might be a risk for public health and economy of the livestock industry in Mongolia.

In chapter 2, a total number of 1,078 goats and 882 sheep blood samples were collected from 17 of 21 provinces and capital city of Mongolia. The seroprevalence of *T. gondii* IgG antibodies was determined by an iELISA based on recombinant antigens of dense granule protein 7 of *T. gondii*. In the present study, the risk factors of sex, age and location to the seroprevalence were investigated. Overall, the seroprevalence of *T. gondii* among the goat and sheep samples was 32% and 34.8%, respectively. The seroprevalence among goat samples was significantly higher in western (42.7%) and eastern (45.6%) regions compared with other regions (24%). Additionally, the seroprevalence among

sheep was significantly higher in eastern regions (55.4%) compared with other regions (26%–33%). Age, but not sex, was considered a risk factor for T. gondii seropositivity in goats, whereas no statistically significant differences were observed in sheep for age or sex. I found the highest seroprevalence of *T. gondii* in sheep and goat in 1-2 years old animals compared with 3-4 and 5-6 years old animals. Especially, the seroprevalence in 1-2 year-old goat was statistically higher than that in 3-4 and 5-6 years old animals. In my study, higher distribution of *T. gondii* was confirmed in goats and sheep, indicating that they are susceptible hosts of *T. gondii* infection. Therefore, this higher seroprevalence of *T. gondii* might affect livestock mortality.

In chapter 3, four species of actinomycetes were isolated from soil samples in Mongolia. Phylogenetic analysis of these isolates revealed that they shared the highest similarity with *Streptomyces canus* (N25), *S. cirratus* (N6), *S. bacillaris* (N18) and *S. peucetius* (N12), based on 16S rRNA gene sequencing. Crude extracts obtained using ethyl acetate were separated by thin layer chromatography. The fractions were then evaluated for their cytotoxicities and their anti-*Toxoplasma* and antimalarial activities *in vitro*. The individual crude extracts from N12 and N6 did not inhibit parasite growth *in vitro* (>100 µg/ml). The crude extract from N18 was able to inhibit parasite growth (IC_{50}: 91.8 ng/ml for *T. gondii*, IC_{50}: 156.9 ng/ml for *P. falciparum* IC_{50}: 316 ng/ml for HFF cells). However, the cytotoxic effect of the N18 extract on the host cells prohibited further assessment of its antiprotozoal activity (Selective index (SI): 3.4 for *T. gondii*, SI: 2.0 for *Plasmodium falciparum*). I also evaluated the efficacies of some well-known antibiotics (e.g., amphomycin, kanamycin, and resistomycin) against *T. gondii* and *P. falciparum in vitro*, which are derived from the identified strains. The antibiotic amphomycin was also found to exhibit antimalarial activity against *P. falciparum* (IC_{50}: 9.3 µM), without any general cytotoxicity being observed. This is the first report on the antimalarial activity of amphomycin. The *S. canus* (N25) crude extract was selected for further chemical characterization based on its antiprotozoal activities because of its lower cytotoxicity (>100 µg/ml) and antiprotozoal activities (IC_{50}: 19.2 µg/ml for *T. gondii*, IC_{50}: 4.9 µg/ml for *P. falciparum*) was fully evaluated chemically. Using liquid chromatography-high

resolution mass spectrometry, phenazine-1-carboxylic acid (PCA) was detected and identified in the active fractions of the metabolites from strain N25. I next confirmed that commercially available PCA possesses antiprotozoal activity against *T. gondii* (IC_{50}: 55.5 μg/ml) and *Plasmodium falciparum* (IC_{50}: 6.4 μg/ml) *in vitro.* The present study is the first to report on PCA being identified in *S. canus* to date. The results of this study reveal that soil actinomycetes are potential sources of antiprotozoal compounds, and that PCA merits further investigation as an anti-protozoal agent.

My current study demonstrated high seroprevalences of *T. gondii* and *N. caninum* in livestock in Mongolia which provides new data for development of control measures against these infections. Additionally, my results suggested that *S. canus* isolated from Mongolian soil may be a potential source of new treatments for human parasitic infections.

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2. Abstract should be between 1,800 and 2,200 characters in Japanese, or be between 1,000 and 1,400 words in English.

3. Do not include figures and tables.

4. Abstract can be longer than one page.