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Abstract of Thesis/Dissertation

Applicant

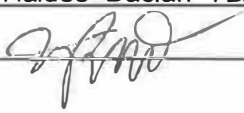
Master's/Doctoral Program in Animal and Food Hygiene

Graduate School of Animal Husbandry

Obihiro University of Agriculture and Veterinary Medicine

Student ID: 17360002

Name of Applicant: Rochelle Haidee Daclan YBAÑEZ

Signature of Applicant: 

Title : Seroepidemiological study of *Toxoplasma* infection in the Philippines and the application of an immunochromatographic test for its diagnosis

(フィリピンにおけるトキソプラズマ感染の血清疫学調査と
イムノクロマトテストの診断への応用)

Abstract

Toxoplasmosis is considered one of the world's most widely distributed parasitic diseases. It is caused by *Toxoplasma gondii,* a ubiquitous single-celled intracellular apicomplexan parasite. *T. gondii* can infect humans and practically all warm-blooded animals, but it is mainly transmitted through the ingestion of bradyzoites-containing tissue cysts in the infected animals and oocysts shed by an infected cat that acts as its definitive host. Toxoplasmosis affects approximately one - third of the world's human population. But it is generally asymptomatic in immunocompetent individuals, or it may only manifest flu-like symptoms and other non - specific clinical signs. However, the public health importance of toxoplasmosis is recognized worldwide because of its zoonotic potential to cause human abortion, stillbirth, and fetal abnormalities. It has been associated with mental and behavioral changes in humans. Acute infection of *T.*

gondii is also potentially fatal in immunocompromised individuals such as HIV patients. Considering the health threat it poses, prompt and accurate detection of *T. gondii* infections is vital. Assessing its presence using the appropriate diagnostic tools can be the first step in determining its relevance to a locality for proper intervention.

In Chapter 1 of this study, the *Toxoplasma* seroprevalence in pigs, humans, and cats in Cebu, Philippines, was evaluated. *T. gondii* infection was reported to be detected in pigs about three decades ago. A total of 924 humans, 104 cats, and 514 slaughter pigs were tested for antibodies against *T. gondii* using a commercial latex agglutination test (LAT). The results revealed positive detection rates of 26.3% (244/924) for humans, 42.3% (44/104) for cats, and 13.4% (69/514) for slaughter pigs. Statistical analyses revealed that the area ($P = 0.004$), cat ownership ($P = 0.020$), the frequency of contact with cats ($P < 0.0001$), and consumption of street foods ($P = 0.043$) were significantly associated with seropositivity for *T. gondii* in humans. Meanwhile, the use of litter trays ($P = 0.001$) and contact with other animals ($P = 0.007$) were significantly associated with seropositivity in cats. The odds ratio for selected significant factors revealed that living in suburban areas (OR 1.66, 95% CI: 1.20–2.31), owning a cat (OR 1.482, 95% CI: 1.07–2.07) and eating street foods (OR 1.585, 95% CI: 1.01–2.48) were associated with an increased risk of *T. gondii* exposure in humans. In cats, the use of a litter tray (OR 4.5, 95% CI: 1.73–11.71) was associated with an increased risk of exposure. None of the profile parameters were found to be significantly associated with seropositivity in slaughter pigs ($P > 0.05$). This study reports the first serological detection of *T. gondii* in humans and cats in Cebu, Philippines, and the first assessment of the prevalence of the parasite in pigs in the area since its initial detection in 1982. To the best of my knowledge, this is also the first report documenting the seropositivity of *T. gondii* in

pregnant women in the country. The confirmed seropositivity of *T. gondii* in Cebu, Philippines, in this study implies the endemicity of toxoplasmosis in this area and highlights the need for routine testing and increased public awareness.

T. gondii secretes huge quantities of dense granule antigens (TgGRAs), which are fundamental to the survival of the parasite. The TgGRA7 is found abundantly on the surface and cytosol of host cells, and within the parasitophorous vacuole lumen and membrane. It is also expressed in all infectious stages of *T. gondii.* TgGRA7 proteins expressed in tachyzoites and bradyzoites are extracellularly released after the rupture of host cells, allowing direct contact of the antigens with the host immune system. This eventually stimulates a strong antibody response during acute and chronic infections. While it has been well utilized as an antigen for enzyme-linked immunosorbent assay (ELISA), only one report has documented its efficacy as an antigen for immunochromatographic test (ICT) in pigs. To date, there is no study yet documenting its use for ICT serodiagnosis of *T. gondii* infection in humans and cats. Hence, the corresponding potential of TgGRA7 as an antigen was evaluated in the proceeding studies.

In Chapter 2, the efficacy of the developed TgGRA7-ICT was validated by testing 88 human serum samples. Its results were compared with those obtained by indirect ELISA (iELISA) based on TgGRA7, a commercial ELISA, and LAT. The TgGRA7-ICT results revealed an excellent agreement with standard test results, as shown in its high sensitivity, specificity, and kappa values. A strong correlation between the relative ICT band intensity and absorbance values in the iELISA was also obtained. Altogether, the data suggest that the current ICT with TgGRA7 is a reliable test for the diagnosis of human toxoplasmosis, which produced results similar to conventional

serological methods. Thus, this can be used as a screening tool for routine testing of toxoplasmosis and a good option for point-of-care application. This study is the first report on the use of TgGRA7 as an ICT antigen for the serodiagnosis of human toxoplasmosis.

In Chapter 3, the usefulness of theTgGRA7-ICT was assessed for the detection of *Toxoplasma* infections in 100 cats. Its results were compared with that of the iELISAs using TgGRA7 and lysate antigens of *T. gondii* strains, RH, PLK, and VEG. The high sensitivity, specificity, and kappa values obtained in this study revealed that TgGRA7-ICT is a reliable test for the diagnosis of anti-*T. gondii* antibodies in cats, which produced comparable results with the conventional serological methods. This study is also the first report on the use of TgGRA7 as an ICT antigen for the serodiagnosis of *T. gondii* infection in cats.

The ultimate goal of this research endeavor was to develop serodiagnostic assays for *T. gondii* infection that can be suitable for a nationwide epidemiological and point-of-care application. This goal was achieved by conducting human and animal epidemiological studies using current serodiagnostic tools to establish baseline information and identify associated risk factors for intervention in the Philippines, and by evaluating the application of TgGRA7 as an antigen for *T. gondii* serodiagnosis using ICT in humans and cats. The approach adopted in this research supports the development of TgGRA7 for ICT in assessing *T. gondii* infection on a large scale.