

NRCPD-OUAVM Joint Research Report

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Project no: 2025-joint-16

1. Principal investigator NRCPD Joint Research Fellow

Name: María Cecilia Venturini.

Institution: Faculty of Veterinary Sciences/La Plata National University – (FCV-UNLP)

Position: Head Professor (period 2025/ February 2026)

2. Project title:

Comparison study of the whole genome sequencing of Toxoplasma gondii strains isolated from Argentina and Japan

3. Collaborating research group members at NRCPD

PI: principal investigador

First name: Juan Manuel. Family Name: Unzaga

Position: Professor

Affiliation: Faculty of Veterinary Sciences, La Plata National University. Argentina

Co-I

First name: Lais Luján. Family name: Pardini

Position: Associate Professor. Researcher CONICET

Co-I

First name: Mariana. Family name: Bernstein

Position: Researcher CONICET

Counterpart investigator at NRCPD Yoshifumi Nishikawa

4. Research period

April 1, 2025, ~ March 31, 2026. Total: 2025-2027

5. Purposes and objectives

Toxoplasmosis is a worldwide zoonotic disease caused by the parasite *Toxoplasma gondii*, which can induce different types of lesions depending on the host species and the immune response. A wide diversity of non-clonal genotypes has been identified in South America. Whole genome sequencing (WGS) analysis may provide further information on Argentine strains and allow comparative analyses

with Japanese strains contributing to a better understanding of the phylogeny, epidemiology, and genetic markers associated with parasite virulence. This project established an initial collaboration with Dr. Nishikawa's laboratory through a three-year collaborative study focused on:

- the evaluation of the virulence of non-clonal Argentine isolates obtained from different geographic areas, and
- the development of affordable diagnostic kits for toxoplasmosis

6. Outline of research process

Isolates were obtained and genetically characterized using a multilocus nPCR-RFLP approach based on 10 standardized genetic markers. Samples were collected from different Argentine provinces (Buenos Aires, Misiones, Entre Ríos, and San Luis) and included:

- Free-range chickens (environmental sentinels)
- Cats, pigs, goats, and other domestic animals
- Wildlife and zoo animals
- Human cases, including congenital infections

During this initial year (April 2025 – March 2026), the most relevant *Toxoplasma gondii* isolates were selected. DNA samples were extracted at LAINPA and subsequently sent to the National Research Center for Protozoan Diseases (NRCPD) for WGS analysis.

Approximately 5 µg of DNA from each strain were obtained from VERO cell cultures under standard conditions. Tachyzoites (10^8) were purified by filtration, and DNA was extracted using a commercial kit (InbioHighway). DNA samples were stored at 4 °C until shipment.

DNA from the isolates TgHm17-1Arg (ToxoDB #14) and TgHm12-1Arg (ToxoDB #285), obtained from cases of congenital human toxoplasmosis in Buenos Aires Province, as well as TgCk11-9Arg (ToxoDB #19) from a chicken in Misiones Province and TgMy1Arg (ToxoDB #163) from a squirrel monkey in Buenos Aires Province, was sent to Dr. Nishikawa's laboratory.

DNA concentration was measured using Qubit dsDNA BR (Thermo Fisher). Libraries were prepared with the Illumina DNA Prep kit using a minimum of 100 ng of DNA, and sequencing was performed on the Illumina NextSeq 1000 platform using SBS chemistry. Preliminary results are currently under analysis.

Future analyses will include the isolates TgCkC24Arg (#123), TgCkP22Arg (clonal type II), TgWb1Arg (#14), TgCk13-5Arg (#116), TgCk14-5Arg, TgCk14-6Arg (#283), and TgCk14-7Arg (#283). The inclusion of these strains will expand the available genomic information on Argentine isolates and further support the molecular characterization of relevant genetic variants and SNPs potentially associated with differences in the biological behavior and pathogenicity of the parasite.

To this end, DNA shipment to Japan is planned in two batches during 2026. DNA samples will be prepared as previously described.

7. Outline of research achievements

This collaborative project has strengthened scientific cooperation in toxoplasmosis research between Argentine and Japanese research groups.

Between March 9 and March 23, 2026, the principal investigator of the research group, Dr. Juan Manuel Unzaga visited NRCPD, where he delivered two lectures on March 13 and 17, presenting recent studies conducted by the LAINPA research group, Faculty of Veterinary Sciences, National University of La Plata (FCV, UNLP), La Plata, Argentina.

The lectures presented were:

- **Advances in Toxoplasmosis Research at LAINPA–Argentina:**

Genetic diversity and population structure of Toxoplasma gondii in animal and human isolates in a South American context

- **LAINPA–Argentina: Integrality and Co-Creation in Territorial Intervention and Science Communication**

In addition, Dr. Unzaga's visit provided an opportunity to meet the research team and become familiar with the research facilities and working environment of the NRCPD. This experience highlighted the importance of continuing the exchange of LAINPA researchers during the next two years of the collaborative project.

As part of this collaboration sequencing and preliminary bioinformatic analyses were performed in Dr. Nishikawa's laboratory, generating approximately 24 Gb of sequencing data, including two reference strains, using the Illumina NextSeq 1000 platform. Future analyses will include ancestry inference and population structure analysis based on SNP and CNV polymorphisms using the POPSICLE software. In addition, principal component analysis (PCA) and phylogenetic network analyses will be performed to investigate evolutionary relationships and identify shared genomic regions and variants.

8. Publication of research achievements

The achievements derived from this collaborative project will be presented at scientific meetings and conferences, and the related works are currently under preparation.