

## NRCPD-OUAVM Joint Research Report

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### 1. Principal investigator

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Position: Associate Professor

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### 2. Project title:

Molecular detection of tick-borne pathogens in Cattle from different altitudes of Nepal

### 3. Collaborating research group members at NRCPD

Name: Masahito Asada

Position: Associate Professor

### 4. Research period (in mm/dd/yyyy, and total number of years)

04/01/2025 – 03/31/2026, one year

### 5. Purposes and objectives

The purpose of this study is to investigate the prevalence, and distribution of tick-borne pathogens (TBPs), with particular emphasis on *Babesia* and *Theileria* species, among cattle reared at different altitudinal in central part of Nepal. Tick-borne diseases represent a major challenge to livestock production and animal health, especially in rural and resource limited farming communities where access to veterinary services, diagnostic facilities, and disease control measures is often inadequate. These pathogens can cause significant economic losses through reduced milk production, weight loss, anemia, and increased mortality in affected cattle. The study aims to assess the epidemiological status of TBPs across varying ecological and climatic conditions associated with altitude. Both microscopic examination and molecular method were employed to detect, identify, and characterize the TBPs in cattle populations. Furthermore, this research generated baseline epidemiological data on the distribution of *Babesia* and *Theileria* infections in central Nepal, where information regarding TBPs remains limited.

## 6. Outline of research process

The study was conducted in three districts of central Nepal, Chitwan, Kaski, and Mustang District, representing low, mid, and high altitude. The study area covered an altitudinal range of approximately 200 to 4,000 meters above sea level. This study provides the first molecular evidence of TBPs in cattle across different altitude in Nepal. Approximately 3 mL of blood was drawn from the jugular vein of each cattle using sterile syringes and placed in EDTA vials. Thin blood smears were prepared immediately after sample collection, fixed with methanol, and stained with Giemsa stain for microscopic examination. The stained smears were observed under a light microscope for the detection of TBPs. For molecular analysis, genomic DNA was extracted from selected blood sample from commercially available DNA extraction kit. The extracted DNA was then subjected to PCR assays to detect the presence of specific TBPs, particularly *Babesia* and *Theileria* species. PCR-positive samples were further subjected to sequencing analysis to confirm pathogen identity.

## 7. Outline of research achievements

Cattle blood samples were collected from both the lowland Terai region of Chitwan and the hilly region of Kaski, while sample collection from the Mustang, mountain region is still in progress. In Chitwan, a total of 50 blood samples were collected, including 18 samples from Jersey and 32 samples from Holstein cattle. Among these, 7 samples were found positive through microscopic examination. These positive samples were further subjected to PCR analysis, and all were confirmed positive for piroplasma infection. Similarly, 191 cattle blood samples were collected from Kaski district. At present, samples from both study areas are undergoing microscopic examination and molecular analysis to detect TBPs. Similarly, hematological parameters were collected and it will also be analyzed and compared between TBPs-positive cattle and healthy cattle to determine the correlation between TBPs infection and changes in hematological profiles.

Besides the above study, microscopy and molecular analyses were also performed in the Terai region near Chitwan, particularly in Nawalparasi West, a district located in Lumbini Province. It lies at an altitude ranging from 100 to 1,491 meters above sea level. The cattle selected for the study were reared under confined management systems. A total of 100 samples were collected from 10 selected farms in different wards of Bardaghat Municipality of Nawalparasi West. Microscopic examination revealed 7% (7/100) positivity for TBPs. Fifty samples were DNA extracted and PCR analysis was performed. PCR analysis identified 50% (25/50) samples as positive TBPs from Bardaghat Municipality.

The manuscript from our previous year's research work has already been submitted to a journal and is currently under revision. Similarly, a manuscript based on the current study will also be prepared and will be submitted in the near future. In addition, the author has ongoing research

collaboration with Dr. Asada on other parasitic diseases, and recently a research paper on toxoplasmosis was successfully published.

## 8. Publication of research achievements

Thapa M, Gombo TR, Sugi T, **Asada M**, Arima H, **Pandey K** (2025). Seroprevalence and associated behavioral factors of *Toxoplasma gondii* infection among pregnant women in Pokhara Valley, Nepal. Tropical Medicine and Health. 53(1): 128. doi:10.1186/s41182-025-00803-8

Attach reference materials as necessary.

➤ Trop Med Health. 2025 Sep 30;53(1):128. doi: 10.1186/s41182-025-00803-8.

# Seroprevalence and associated behavioral factors of *Toxoplasma gondii* infection among pregnant women in Pokhara Valley, Nepal

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## Abstract

**Background:** *Toxoplasma gondii* is a protozoan parasite that can cause congenital infections with serious health implications. In pregnant women, *T. gondii* infection poses significant risks, including miscarriage, stillbirth, and congenital abnormalities in the fetus. The burden of toxoplasmosis is often underrecognized in many developing countries, including Nepal, where awareness and routine screening are limited. This study was aimed at determining the prevalence of *T. gondii* infection among pregnant women in Pokhara Valley, Nepal, and to identify behavioral risk factors associated with the transmission of this parasite.

**Methods:** A cross-sectional study was conducted at Gandaki Medical College Teaching Hospital, Pokhara Valley, Nepal, from October 2024 to January 2025. A total of 257 serum samples were collected, 91 of which were randomly selected and tested for anti-*T. gondii* IgG/IgM antibodies using a rapid diagnostic test (RDT) and enzyme-linked immunosorbent assay (ELISA). Sociodemographic