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 characterization and genetic diversity of tick-borne diseases in 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)

01/04/2023 -31/03/2024: one year

5. Purposes and objectives

Tick-borne diseases cause significant morbidity and mortality in animals in Nepal. Piroplasm is one of the important protozoan parasites in the cattle. There have been very few studies about tick-borne parasitic diseases in Nepal. Those studies were focused on the detection of tick-borne parasites using microscopy. There were no molecular studies on tick-borne parasites in Nepal. The objective of this study was to detection of tick-borne parasites in cattle in Nepal using molecular methods.

6. Outline of research process

The blood samples were collected from cattle in three districts (Kathmandu, Lalitpur, and Bhaktpur) of Kathmandu Valley, Nepal. Thin blood smears were prepared, fixed with methanol, and stained with Giemsa solution. The smears were observed under a microscope for the detection of blood parasites. Then, DNA was extracted from each cattle blood sample, followed by PCR targeting piroplasm-specific primers. PCR-positive samples were further confirmed by sequencing.

7. Outline of research achievements

The study was conducted to detect the types of piroplasm affecting cattle in the Kathmandu Valley, Nepal using molecular methods. In this hospital-based study, 106 blood samples were collected from cattle that were brought to the hospital for proper diagnosis of blood-borne disease infections. Thin

blood smears were prepared and observed under the microscope. Similarly, blood samples were used for DNA extraction. PCR and sequencing were performed to detect and identify piroplasm present in the collected samples. This study revealed that 45 (42.5%) were positive for piroplasm (*Babesia* spp. and *Theileria* spp.) via microscope observation and 56 (52.8%) samples were positive via PCR. We were able to identify the species as *B. bigemina, B. bovis*, *T. annulate* and *T. orientalis* by PCR followed by sequencing. Nine samples were selected and performed for sequencing. The phylogenetic analyses of those sequenced samples showed that the *B. bovis, B. bigemina* and *T. orientalis* sequences belonged to each species clade. On the other hand, *T. annulate* was divided into two clades in the analysis, and our *T. annulate* sequences were also divided into these two clades. The result is the first to detect piroplasm using PCR techniques in Nepal.

8. Publication of research achievements

Dhakal M, Gompo TR, Devkota P, Kafle SC, Subedi JR, Gong H, Arima H, Culleton R, **Asada M, Pandey K.** Molecular detection and Identification of piroplasm in cattle from Kathmandu Valley, Nepal. Pathogens. 5;12(8):1045.

Attach reference materials as necessary.

› Pathogens. 2023 Aug 15;12(8):1045. doi: 10.3390/pathogens12081045.

Molecular Detection and Identification of Piroplasm in Cattle from Kathmandu Valley, Nepal

Medhavi Dhakal [1], Tulsi Ram Gompo [2], Prakash Devkota [2], Sharmila Chapagain Kafle [2], Janak Raj Subedi [1], Haiyan Gong [3], Hiroaki Arima [4], Richard Culleton [5], Masahito Asada [6], Kishor Pandey [1]

Affiliations – collapse

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