NRCPD-OUAVM Joint Research Report

Date: 2025.5.27

Project no: 2024-joint-17

1. Principal investigator

Name: Kishor Pandey

Position: Associate Professor

Affiliation: Central Department of Zoology, Tribhuvan University, Institute of Science and Technology, Kathmandu, Nepal

2. Project title:

Molecular detection of tick-borne pathogens in Cattle from Kathmandu, 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)

04/01/2024 – 03/31/2025, one year

5. Purposes and objectives

The purpose of this study is to investigate the presence and distribution of tick-borne pathogens (TBPs) mainly *Babesia* and *Theileria* species in cattle within Dolakha District, Nepal. TBPs pose a significant threat to livestock health and productivity, particularly in rural areas where veterinary resources may be limited. The objective of the study is to utilize molecular techniques to detect and identify the TBPs transmitted by ticks affecting cattle in this region. This research aims to provide baseline data that can support effective disease management strategies, improve animal health, and contribute to the development of targeted control and prevention programs in Nepal.

6. Outline of research process

The study was conducted at Cattle Genetic Resource Centre (CGRC) which is situated at the Jiri Valley, Dolakha district, Nepal at altitude of 1935 meters above sea level. The study area is located in the Himalayan region which has a cooler climate and diverse topography, which influences tick infestation and disease prevalence. These cattle graze on open pasture land near the CGRC. The blood samples were collected from each cattle in sterile conditions. Three ml blood was taken from jugular vein of each cattle. Thin blood smears were prepared, fixed with methanol, and stained with Giemsa. The smears were observed under a microscope for the detection of TBPs. DNA was extracted from

each blood sample and subjected to PCR analysis to detect the presence of specific TBPs. Positive samples will be further analyzed through sequencing to confirm pathogen identity.

7. Outline of research achievements

The study was conducted to detect the types of tick-borne pathogens (TBPs) affecting cattle farm at himalayan region of Nepal, Nepal using molecular methods. A total of 80 blood samples were collected from cattle at CGRC, Dolakha, Nepal in year 2023 and 2024. Thin blood smears were prepared and observed under the microscope. DNA was extracted using commercial DNA extraction kit. PCR, cloning and sequencing were performed to detect and identify TBPs present in the collected samples. This study revealed that 66 (77.5%) were positive for piroplasm (*Babesia* spp. and *Theileria* spp.) via PCR. We were able to identify the species as *B. bigemina, B. bovis*, and *T. orientalis* by PCR followed by sequencing. The sequencing revealed distinct nucleotide sequences of *Babesia* in 42 samples. Specifically, *B. bovis* was seen in 20 samples and *B. bigemina* was seen in 22 samples. Similarly, 10 *T. orientalis* were seen in 10 samples. Thirteen samples were clone to see co-infection and result showed 2 samples showed *B. bovis* and *B. bigemina* co-infection. Similarly, one sample showed *B. bovis* and *T. orientalis*. The result is the first to detect co-infection of two parasites using sequencing of clone samples in Nepal.

In addition, the author has collaboration with Dr. Asada in other parasites and recently we published paper on canine demodicosis.

8. Publication of research achievements

Bhusal R, Gompo TR, Sugi T, **Asada M, Pandey K (2025)**. Canine Demodicosis in Rupandehi Nepal's Street Dogs: Prevalence, Clinical Signs, and Hematology. Vet Sci. 12(3):238. doi: 10.3390/vetsci12030238.

> Vet Sci. 2025 Mar 3;12(3):238. doi: 10.3390/vetsci12030238.

Canine Demodicosis in Rupandehi Nepal's Street Dogs: Prevalence, Clinical Signs, and Hematology

Rachana Bhusal [1], Tulsi Ram Gompo [2], Tatsuki Sugi [3], Masahito Asada [4], Kishor Pandey [1]

Affiliations – collapse

Affiliations

1 Central Department of Zoology, Institute of Science and Technology, Tribhuvan University, Kathmandu 44601, Nepal.
2 Central Veterinary Laboratory, Kathmandu 44600, Nepal.
3 Division of Collaboration and Education, International Institute for Zoonosis Control, Hokkaido University, Sapporo 001-0020, Japan.
4 National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obhiro 080-8555, Japan.

PMID: 40266949 PMCID: PMC11946785 DOI: 10.3390/vetsci12030238

Abstract

Canine demodicosis is a contagious skin disease caused by the over-proliferation of *Demodex* mites in the host's hair follicles. This study examines the prevalence, clinical signs, and hematological changes associated with demodicosis in street dogs of Rupandehi, Nepal. Between August 2023 and January 2024, 100 skin scrapings were collected from each street dog presenting dermatological symptoms.