NRCPD-OUAVM Joint Research Report

Date: 2024.5.24
Project no: 2023-joint-18

1. Principal investigator

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2. Project title:

Genetic diversity of *Theileria equi* infecting equines in India and quantification of parasite loads

3. Collaborating research group members at NRCPD

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Position: 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

Theileriosis is an economically important, tick-transmitted protozoan disease of livestock. The disease can cause high mortality rates of 70% to 80% in susceptible animals. In India, equine theileriosis caused by *Theileria equi* is widespread, causing severe economic losses. Therefore, control of equine theileriosis is vital in this country. However, lack of understanding the role of carrier animals in the *T. equi* epidemiology is a stumbling block for designing the control strategies in India. Despite the low parasitaemia in latently infected equines, *T. equi* can be tick-transmitted from these carrier animals to naïve animals, where the infection may result in severe clinical theileriosis. Therefore, detecting the carrier animals and estimating the parasite loads are important for the risk assessment and effective management of equine theileriosis, using the available resources in India.

The strategies to control equine theileriosis will be more effective, if they consider the genotypic diversity of *T. equi*. In common with other hemoprotozoan parasites, *T. equi* is genetically diverse, and consists of five genotypes, including genotypes A-E. The merozoite surface antigens, which have been frequently used to develop several diagnostic assays, are highly diverse among the *T. equi* genotypes, sometimes leading to false negative diagnostic test results. For example, the EMA-1, based molecular and serological assays, is found in the genotype A, but not in the genotype C. Therefore, assessment of the genetic diversity of *T. equi* is important for selecting diagnostic assays in the endemic countries. Additionally, the genotype A of *T. equi* is reported to be more commonly associated with clinical theileriosis, as compared to the other genotypes. Similarly, recent studies found that repeated

treatment with anti-theilerial drugs may clear the genotype A from the infected horses, but not the genotype C. Therefore, a comprehensive understanding of *T. equi* genetic diversity is vital for designing effective disease control programs. In India, however, the genetic diversity of *T. equ*i has not been fully investigated, rendering the control methods less effective.

Therefore, the proposed study has been designed to investigate the genetic diversity of *T. equi* infecting equines in India and to determine the parasite loads in equines with latent infection.

Objective

To study genetic diversity of *Theileria equi* infecting equines in India and quantify the parasite loads in carrier animals using a qPCR assay.

6. Outline of research process

Materials and Methods

Blood sampling: Blood samples were collected from 84 equines bred in two Indian states, including Haryana (n=28) and Rajasthan (n=56), into EDTA-coated vacuum tubes. DNAs were extracted from the blood pellet using a commercial kit. From each animal, blood samples were also collected into plain tubes without any anti-coagulants, and sera were obtained. Both the DNA and serum samples were then stored at -20°C untill further use.

ELISA: An EMA-2-antigen-based ELISA that we had developed in our laboratory at the NRCE was used to screen all 84 sera for detecting antibodies against *T. equi* (Kumar et al., 2013. Vet Parasitol. 198, 10-7.). Final ELISA OD_{492} cutoff point was determined by calculating the relative percent positivity (RPP). Samples showing RPP >20 considered as positive.

PCR detection of *T. equi* and its genotypes: All of the 84 equine blood DNA samples were initially screened using a *T. equi*-specific PCR assay (Alhassan et al., 2007. Vet Parasitol. 143, 155-60.). The positive samples were further analyzed with PCR assays specific to genotype A – E (Ahedor et al., 2023. Parasit & Vectors. 16, 435.).

7. Outline of research achievements

Of 84 samples tested, 42 (50.0%) were positive for *T. equi*-antibodies with RRP values ranged from 67 % to 189%. On the other hand, 27 samples (32.1%) were positive for *T. equi* infection in the screening PCR assay. These 27 *T. equi*-positive samples were further analyzed with the genotype-specific PCR assays. We found that the surveyed equines were infected with the genotypes A and D of *T. equi*. In brief, 7 and 5 equines had single infections with genotypes A and D, respectively, while the remaining 15 were co-infected with the genotypes A and D. The present study is the first to report the *T. equi* genotype D in India, highlighting the potential challenges associated with the management of equine theileriosis. With the results obtained from this collaborative research, we are currently in the process of cultivating the genotype D *in vitro* to explore its diagnostic, clinical, and therapeutic implications.

8. Publication of research achievements

In Progress

Attach reference materials as necessary.