

# NRCPD-OUAVM Joint Research Report

Date: May 9<sup>th</sup>, 2020

Project no: 2019-joint-18

## 1. Principal investigator

Name: Haiyan Gong

Position: Associated professor

Affiliation: Shanghai Veterinary Research Institute, Chinese Academy of Agricultural Sciences

## 2. Project title:

Microbiome comparison of the tick *Haemaphysalis longicornis* from the labs of China and Japan

## 3. Collaborating research group members at NRCPD

Name: Rika Umemiya-Shirafuji

Position: Assistant Professor

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

April 1, 2019 - March 31, 2020, one year

## 5. Purposes and objectives

- (1) To obtain pathogenic microbiota in *H. longicornis* in two labs, which hints the potential infection that we should consider in the future.
- (2) To compare the composition of microorganisms harbored by unfed adult ticks cultured in the lab of Shanghai Veterinary Research Institute (SHVRI), Chinese Academy of Agricultural Sciences (CAAS) and NRCPD, so that we can cooperated with each other to deal with the common potential pathogens.

## 6. Outline of research process

- (1) Nymphs of *H. longicornis* were fed on SPF New Zealand White rabbits until they were engorged and developed into adults. Five adults are washed with 75% ethanol twice and distilled water three times. Each adult tick was considered as one sample.
- (2) Five whole tick bodies were extracted for DNA respectively. The quantity and quality of extracted DNAs were measured using spectrophotometer and agarose gel electrophoresis, respectively.
- (3) The DNA from each sample is used as template for amplification of V5-V6 region of the 16S rRNA gene, followed by pyrosequencing on an Illumina MiSeq platform.
- (4) The obtained data from both labs are compared for the differences and similarities on the pathogens and symbionts.

## 7. Outline of research achievements

- (1) Even though the ticks came from the same lab, the microbiota in the tested 5 ticks of China was still different from each other. Only 174 genera of microorganisms were detected in HLLab3, while 354 genera were found in HLLab4.
- (2) As the symbionts in ticks, *Coxiella* showed an extreme proposition in *H. longicornis* from the lab of China as well as that of Japan. It is putative that ticks experienced a deletion of pathogens in the environment of labs. In the present study, tick-borne pathogens were not detected in the top 20 genera, which is obviously different from the field samples. As our previous other study, *Rickettsia. spp* was massively detected in the field ticks from Qinghai, Shanxi and Yunnan province of China (data was not published yet). However, the field samples were half-fed or engorged ticks, which may include the pathogens in the blood of the hosts.
- (3) Five ticks from the same clone demonstrated much difference on microbiome, but they still shared about 69 ASV/OTUs in common. The data was compared with the microbiota that harbored by ticks reared in NRCPD of Japan, and it was found that more than 93% of the sequencing reads were identical, which were assigned to *Coxiella sp.* (Umemiya-Shirafuji et al., unpublished data).

## 8. Publication of research achievements

The data about the microbiome of cultured *H. longicornis* in Chinese lab has been shared with assistant professor Rika Umemiya-Shirafuji. And the paper on the comparison of tick harbored microorganisms from both labs is under submission.

Attach reference materials as necessary.