

## WILD DEER AS RESERVOIRS OF EMERGING TICK-BORNE DISEASES OF HUMANS AND DOMESTIC ANIMALS

Hisashi INOKUMA<sup>1</sup>, Takako FUJIMOTO<sup>1</sup>, Li LI<sup>1</sup>, Eiji HOSOI<sup>1</sup>, Shuhei TANAKA<sup>1</sup>, Kozo FUJISAKI<sup>2</sup>, Masaru OKUDA<sup>1</sup> and Takafumi ONISHI<sup>1</sup>

1) Faculty of Agriculture, Yamaguchi University, 1677-1 Yoshida, Yamaguchi 753-8515, Japan

2) National Research Center for Protozoa Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido 080-8555, Japan

### Introduction

Ticks can transmit many pathogens, such as protozoa, rickettsia and bacteria to both humans and domestic animals. While wild animals can be reservoirs of such kind of infectious diseases in the field. Recently numbers of wild deer, *Cervus nippon*, are increasing in the mountain area of Japan, because of environmental changes. They are occasionally found in cattle pastures or around villages. As the deer is one of ruminants and known to be a closely related wild animal with cattle, the sensitivity against important tick-borne diseases may be similar to cattle. Deer are also known to be infected with many ticks in the field; however, little information can be available on the tick species found on the wild deer in Japan. In the present study, ticks were collected from deer in Yamaguchi Prefecture for identification, and tick-borne pathogens *Babesia* and *Theileria*, *Ehrlichia* and *Rickettsia*, and *Borrelia* infection were examined from the spleen samples by PCR.

### Materials and methods

August to November 1999, and March to July 2000, 94 deer were killed by hunters in western part of Yamaguchi Prefecture, Japan. A small piece of spleen and a part of skin with hair from cervical region were collected from each deer as soon as possible after killing. Totally 53 spleen and 94 skin samples were obtained. The specimen were send to Yamaguchi University within a cold box kept at 4°C. All infested ticks found on the skin samples were collected and transferred into tubes containing 70% ethanol until identification. Ticks were identified morphologically with taxonomic keys and description paper for individual tick species.

Total DNA was extracted from each deer spleen (10 mg) and stored at  $-20^{\circ}\text{C}$  until used. For the screening of *Ehrlichia* spp, a genus-specific primer set, EHR16SD and EHR16SR, which amplify a 345-bp fragment of 16S rRNA gene were used. For all positive samples of PCR with EHR16SD/EHR16SR, other set of primers CS136F (5'-TTY-ATG-TCY-ACT-GCT-GCK-TG-3') and CS778R (5'-GCN-CCM-CCA-TGM-GCT-GG-3') was used to confirm results of the first PCR. This set of primers amplifies a 650-bp fragment of ehrlichial citrate synthase gene. When any positive results were found in a screening PCR, the sample DNA were also tested with species-specific primers for 5 *Ehrlichia* species: *E. phagocytophila*, *Anaplasma centrale*, *E. muris*, *Ehrlichia* from *Ixodes ovatus*, and *E. chaffeensis* as described elsewhere. For the screening of *Rickettsia* infection, genus-specific primers CS877F and CS1273R, which amplify a 396-bp fragment of rickettsia citrate synthase gene. *Borrelia* infection was examined. Both *Babesia* and *Theileria* were screened with a set of primers, RIB-19 and RIB-20 based on the 18S rRNA sequences. When the positive reaction was found in the screening PCR, the PCR products were purified and nucleotide sequences were determined with direct sequence methods described previously.

## Results and discussion

A total of 2703 ticks were recovered. The numbers of ticks recovered from a deer were maximized on October to November, while minimum numbers were recorded on August. *Haemaphysalis flava* and *H. megaspinosa* were dominant species in autumn and winter, while *H. longicornis* and *H. yeni* were found to be dominant in spring to summer. *Amblyomma testudinarium*, *H. kitaokai*, and *Ixodes ovatus* were also recorded occasionally from deer; however the numbers of these ticks were comparatively lower than other dominant species. This tick species was only found and recorded in Yaku-shima island, southern part of Japan before the present study. It is the first record of this tick species in the main land of Japan. The reason why *H. yeni* was found in Yamaguchi Prefecture is unknown; however, climate changes may be one of the factors for the distribution spread of this tick species.

Tick-borne pathogens can be transmitted specifically by tick species. Both *H. longicornis* and *H. megaspinosa* are known to be important vectors of *Theileria sergenti* to cattle. *H. longicornis* is also a known vector of *Babesia ovata* of cattle and *Rickettsia japonica* of human. *H. flava* can also transmit *R. japonica* and *Ehrlichia muris*. While *Ixodes ovatus* is a known vector of *B. microti*, an emerging babesiosis of human, *R. japonica* and *Borrelia* spp, a causative agent of the Lyme disease of human and domestic animals. In China, *H. yeni* is recently revealed to be a vector of spotted fever

group rickettsia and *Ehrlichia chaffeensis*. We then examined these tick-borne pathogens from spleen samples of deer. There were no positive reaction in screening PCR for *Rickettsia* and *Borrelia*; however, of the 53 samples tested, 9 (17 %) showed positive for *Babesia* and *Theileria* spp. and 18 (34 %) showed positive for *Ehrlichia* spp.

The sequence analysis of the positive PCR products in the *Babesia* and *Theileria* screening, revealed that the DNA found in deer spleen are very close to those of *Theileria sergenti* found in cattle. It is possible that deer can be infected with the pathogen and plays an important role as reservoir of the bovine piroplasmiasis. The sensitivity of *Theileria sergenti* to wild deer should be examined to confirm the phenomenon. The species-specific PCR to detect 5 tick-borne *Ehrlichia* species were performed, because *A.centrale* and *E.phagocytophila* can infect cattle, *E.muris* and *Ehrlichia* detected from *I.ovatus* exist in Japan, and *E.chaffeensis* is transmitted by *H. yeni*. However results of the PCR were all negative. We need more analysis to clarify the *Ehrlichia* infection in deer by sequencing, restriction fragment length polymorphism or isolation of the pathogens for the next step.

**Recently numbers of wild deer are increasing in the mountain area of Japan, and they are occasionally found in cattle pastures or around villages. As they sometimes make contact with human or domestic animals, deer may play an important role as reservoirs of tick-borne diseases, such as *Theileria sergenti* or *Ehrlichia* spp.**