



## Expression profiles of *vitellogenin-1* in *Babesia ovata*-infected *Haemaphysalis longicornis* ticks\*

NARIKO SATO<sup>1</sup>, HIROSHI SUZUKI<sup>2</sup> & RIKA UMEMIYA-SHIRAFUJI<sup>3</sup>

National Research Center for Protozoan Disease, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, 080-8555, Japan

<sup>1</sup> [✉ iverson.nari1227@gmail.com](mailto:iverson.nari1227@gmail.com); <sup>2</sup> [✉ hisuzuki@obihiro.ac.jp](mailto:hisuzuki@obihiro.ac.jp)

<sup>3</sup> [✉ umemiya@obihiro.ac.jp](mailto:umemiya@obihiro.ac.jp); <https://orcid.org/0000-0001-7641-5526>

\*In: Zhang, Z.-Q., Fan, Q.-H., Heath, A.C.G. & Minor, M.A. (Eds) (2022) *Acarological Frontiers: Proceedings of the XVI International Congress of Acarology (1–5 Dec. 2022, Auckland, New Zealand)*. Magnolia Press, Auckland, 328 pp.

Ticks are hematophagous arthropods and have a remarkable ability to transmit various parasites. *Babesia ovata*, a protozoan parasite that causes babesiosis in cattle, is transmitted from parent to offspring in *Haemaphysalis longicornis*. Although transovarial transmission is demonstrated experimentally, little is known about the molecular mechanisms of tick-*Babesia* in that phenomenon. Our previous findings suggest that vitellogenin-2 (Vg-2), a yolk protein precursor synthesized in the fat body and ovary, contributes to the survival of the *Babesia* within the tick body and invasion into the ovary. However, the interaction with the *Vg-1* gene, which is specifically synthesized in the midgut where *Babesia* first parasitizes, is unknown. Therefore, we investigated the expression and function of the *Vg-1* gene in *H. longicornis* infected with *Babesia ovata* and attempt to elucidate the role of the *Vg-1* gene in transovarial transmission. Firstly, it verified if the *Babesia* infection affects the expression of the *Vg-1* gene in the midgut. Parthenogenetic *H. longicornis* were fed with *B. ovata*-infected or no *B. ovata*-infected bovine erythrocytes until engorgement by a semi-artificial feeding system. Engorged ticks were dissected in the preoviposition period between 0–2 days after engorgement (dAE) and the midgut, hemolymph, and ovary were collected from each tick. To determine the *Vg-1* gene expression levels in *B. ovata*-infected and non-infected ticks, total RNA was extracted from the midgut, and used for real-time PCR. Genomic DNA was extracted from the hemolymph and ovary to detect the *B. ovata*  $\beta$ -tubulin gene by the nested PCR. Next, to analyze the function of the *Vg-1* gene in the *B. ovata*-infected ticks, *Vg-1* gene knockdown was conducted by RNA interference (RNAi). Double-stranded RNA (dsRNA) of *Vg-1* was generated using *H. longicornis* midgut cDNA. Each unfed female tick was injected with *Vg-1* dsRNA or the firefly luciferase dsRNA as a control, then *B. ovata*-infected blood was fed by a semi-artificial feeding system. The same as above, the engorged ticks were dissected and the midgut, ovary, and carcass were collected. Total RNA was extracted from the midgut and examined knockdown of the *Vg-1* gene expression by real-time PCR. DNA samples of the hemolymph and ovary from *Vg-1*-knockdown ticks were tested to detect the *B. ovata*  $\beta$ -tubulin gene. The expression levels of *Vg-1* in the midgut of *Babesia*-infected ticks significantly increased at 1 and 2 dAE, suggesting that the expressions of *Vg-1* in the midgut were affected by *B. ovata* infection. The detection rate of *B. ovata*  $\beta$ -tubulin was 17.6% in the ovary at 0–1 dAE and hemolymph at 1 dAE. In the ovary at 2 dAE, the detection rate was the highest percentage (25.0%). *Vg-1* expression levels in *Vg-1* dsRNA-injected ticks were reduced to 87.7%, 93.1%, and 94.0% on 0, 1, and 2 dAE, respectively, compared to the control ticks. There were no significant differences in the detection rates of *B. ovata*  $\beta$ -tubulin between control and RNAi tick samples. To estimate the migration of *Babesia* within the tick body, we are trying to perform real-time PCR using DNA samples of RNAi ticks to detect the *B. ovata*  $\beta$ -tubulin gene quantitatively.

**Keywords:** tick, *Haemaphysalis longicornis*, vitellogenin, midgut, *Babesia*, transovarial transmission