Introduction: *Rhipicephalus microplus* is the main cattle ectoparasite and important biological vector of *Anaplasma marginale*. This bacterium is the etiological agent of anaplasmosis, a disease that affects cattle worldwide and causes serious economic losses to the Brazilian cattle production. The gut cells of the tick-vector are the primary target of this bacterium for colonization. After *Anaplasma* multiplication in the gut, they migrate through the hemocoel to the salivary glands. Transmission of the bacteria to cattle occurs via saliva during the tick feeding. One key point to control this disease is to understand the relationship between pathogen-vector. **Material and Methods:** We used the embryonic cell line BME26 from *R. microplus* as experimental model for infection by *A. marginale*. To characterize tick genes modulated in response to *Anaplasma* infection, suppression-subtractive hybridization libraries (SSH) were constructed from infected and uninfected BME26 cells. Five up-regulated genes were selected for a functional genomics analysis *in vivo*. The RNA interference (RNAi) technique was used in this study to determine the effect of gene knockdown on tick bacteria acquisition and transmission. **Results and Discussion:** *A. marginale* infection was evaluated on gut and salivary glands of silenced ticks during acquisition and transmission feeding. Ticks silenced with the cytochrome c oxidase sub III (COXIII) gene, a respiratory chain enzyme at the inner membrane of mitochondria that contribute to the proton gradient, failed to transmit this pathogen to mammalian host. **Conclusion:** This is the first report showing the success of *A. marginale* blocked transmission by silencing a tick gene. Ticks silenced with dsCOXIII had their gut and salivary glands infected but were not able to transmit *Anaplasma* to naïve cattle. COXIII seems to play an important role on transmission process of *A. marginale* by the vector to the host.

**Key words:** *Rhipicephalus microplus*, *Anaplasma marginale*, suppression-subtractive hybridization, RNA interference, cytochrome c oxidase sub III.

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