TOPIC:
10) Artrópodos vectores de enfermedades de importancia veterinaria
APPROACH:
3) Interacción patógeno-vector

Studies of the dinamics of *Anaplasma marginale* strains in the vector and the mammalian host through genotyping.

Keywords: arthropod-borne diseases; veterinary importance; pathogen-vector interaction; *Anaplasma marginale;* genotyping.

Perez, Agustina E[1], Sarmiento, Nestor F [2], Pertile, Carla[2], Farber, Marisa D[1] & Guillemi, Eliana C[1]

[1] Instituto de Agrobiotecnología y Biología Molecular (IABIMO) INTA - CONICET, Hurlingham, Buenos
Aires, Argentina
[2] Estación Experimental Agropecuaria Mercedes, Instituto Nacional de Tecnología Agropecuaria, Mercedes, Corrientes, Argentina
E-mail address: perez.agustina@inta.gob.ar

Anaplasma marginale is a gram-negative obligate intracellular tick-borne bacterium that infects erythrocytes of ruminants and other mammalian host species from tropical and subtropical world regions. In Argentina, the transmission of *A. marginale* is mainly associated to the one-host tick, *Rhipicephalus microplus.* Although the presence of *A. marginale* DNA was previously confirmed in *R. microplus* larvae by PCR, the transovarial transmission of the bacterium in the tick remains neglected. The aim of the present study was to detect and molecularly characterize *A. marginale* in the bovine and in organs from *R. microplus* engorged female removed from the host, in order to determine the *A. marginale* genotypes involved in the transmission process. For this purpose, blood and tick samples were obtained from a bovine with acute Anaplasmosis at an endemic region in Corrientes province, Argentina. In order to get rid of the host blood meal, we dissected a female engorged tick and extracted the ovaries and salivary glands. DNA was extracted from 400 µl of the bovine blood sample using the ADN PuriPrep-S kit (INBIO Highway) and from the tick tissues using the NucleoSpin Tissue kit (Macherey-Nagel). For *A. marginale* identification, we used a species-specific PCR protocol that amplifies two copies of the *msp1β* gene. We also amplified, cloned and then sequenced the 5' end repetitive region of the *msp1α* gene, broadly used for genotyping *A. marginale* strains. We detected *A. marginale* in the bovine blood sample and in the tick organs. We analyzed 30 clones from the bovine blood sample and 8 clones for each tick organ. We then determined the number and type of tandem repeats for each MSP1α coding region. There was a great diversity of genotypes in the bovine (19) that were formed by different combinations of 20 repeats, with a minimum of one (AR12) and a maximum of 8 (AR9-62-61-AR10-62-62-61-AR14). Engorged females organs showed a fewer number of different genotypes (ovaries: 2; salivary glands: 3) and repeats involved (8). Two genotypes found in the engorged female (1 in salivary glands -AR15-62-62-62-61- and other in ovaries –AR9-62-66-62-61-) were not detected in the bovine host which could be the result of a previous transovarial transmission process of *A. marginale*. The high

number of *A. marginale* genotypes detected in the infected bovine and the reduce number in the tick organs suggest a diverse genotype fitness at least for transovarial transmission. Moreover, these results help to explain how a monoxenic cycle tick as *R. microplus,* could be responsible for the large distribution of anaplasmosis in enzootic regions.