

Midichloria mitochondrii: a novel tick-borne infectious agent?D. Sasser³, C. Bazzocchi¹, M. Mariconti², S. Epis¹, A. Cafiso¹, C. Bandi¹¹DIVET, Università degli Studi di Milano, Milano ²IRCCS Policlinico San Matteo ³Dipartimento di Biologia e Biotecnologie, Università degli Studi di Pavia, Pavia, Italy

Objective: *Ixodes ricinus* is a vector of various pathogens, and the most widespread tick in Europe. *I. ricinus* also harbors the Rickettsiales bacterium *Midichloria mitochondrii*, which is present in 100% females and in ~50% males. Most *M. mitochondrii* bacteria are localized in the cells of the ovary, either in the cytoplasm or within the outer membrane of mitochondria. *M. mitochondrii* is vertically transmitted from the tick mother to the offspring; however horizontal transmission, possibly through the host blood, cannot be excluded a priori. We used a multidisciplinary approach to uncover possible transmission of *M. mitochondrii* to humans and other tick hosts.

Methods: We sequenced the genome of *M. mitochondrii* and we were thus able to identify a putative antigenic protein, the flagellar cap FLID. We expressed FLID in recombinant form and obtained polyclonal antibodies against it. We developed molecular and serological tools to screen for the presence of the Flid gene and of its protein product in various biological samples: PCR and sequencing, qPCR, ELISA, Western blot, immunostaining. We collected samples from *I. ricinus* ticks (rostrum, salivary glands and saliva) as well as from tick-exposed mammals and controls (blood and sera). We used the developed tools to determine the presence of *M. mitochondrii* in these samples.

Results: We detected the presence of *M. mitochondrii* in all examined *I. ricinus* samples, using PCR and immunostaining. We also screened various species of tick-exposed mammals to obtain direct and indirect evidence of the presence of *M. mitochondrii*. Twenty six of 186 blood samples (sheep, horses, dogs and cattle) resulted positive in PCR; 58 of 218 dog sera resulted positive in ELISA.

Finally, we screened human sera from tick-exposed patients and controls with ELISA, and we detected the presence of antibodies against *M. mitochondrii* rFLID protein (58% positivity in tick-exposed patients, 1.2% in controls).

Conclusion: Our studies provide evidence that *M. mitochondrii* is possibly inoculated through the bite of *I. ricinus* to the mammalian hosts. Additional studies are necessary to understand if this novel tick-borne agent is able to multiply within the host and whether it may cause pathological effects.