



V 50 *Ixodes ricinus* ticks from Baden-Wuerttemberg, Germany, carry an emerging subclone of *Francisella tularensis holarctica*

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Francisella tularensis is the causative agent of the zoonotic disease tularemia. Due to its extreme infectivity, *F. tularensis* has been classified as a category A select agent by the CDC. Apart from deer flies, horse flies, and mosquitoes, hard ticks have been described as important vectors and potential reservoirs for *F. tularensis*.

We recently postulated that the state of Baden-Wuerttemberg represents a new endemic focus for *F. tularensis* in Germany. To verify whether ticks are involved in the reported increase of tularemia in this southern part of Germany, 916 *Ixodes ricinus* and 211 *Dermacentor marginatus* and *D. reticulatus* ticks were collected in 2 different locations. Screening for the presence of *F. tularensis* was performed by real-time PCR of the 16S rRNA gene.

In ticks of the genus *Dermacentor*, *Francisella*-like endosymbionts (FLEs) were present. These hamper the identification of *F. tularensis* in ticks as they produce false-positive results in the screening PCR.

Eight *I. ricinus* pools (0.9%) were positive for *F. tularensis* in the screening PCR. Confirmation by 30-bp deletion PCR revealed that the *F. tularensis* DNAs belonged to the subspecies *holarctica*. More precisely, as shown by FtM24 MLVA analysis, they belong to the Franco-Iberian subclone group of *F. tularensis holarctica*.

For phylogenetic analysis, the *lpnA* gene region of all *Francisella*-positive *I. ricinus* pools and 6 *Dermacentor* ticks with positive reactions in the screening PCR was amplified and sequenced. In the resulting neighbor-joining tree, all *Francisella*-positive *I. ricinus* samples cluster with *F. tularensis* and *F. tularensis holarctica*, respectively, while all *Dermacentor* tick samples cluster with FLE sequences. These data confirm the results obtained by PCR and FtM 24 analysis.

This study shows that *I. ricinus* ticks may serve as vectors and/or reservoirs for *F. tularensis* in Germany and that the state of Baden-Wuerttemberg represents an endemic focus for tularemia.