

# Whole Genome Phylogeny of Swiss *F. t. holarctica* Isolates

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## OBJECTIVES

It is well documented that ticks serve as primary vector for *F. tularensis* in many endemic regions. Within the scope of a national survey focusing on the role of ticks as zoonotic vectors for a variety of viral and bacterial pathogens, we assessed the prevalence of *F. tularensis* spp in the Swiss *Ixodes ricinus* tick population.

To describe the genetic and epidemiological dynamics of *F. tularensis holarctica* in Switzerland, strains isolated from ticks were compared to clinical isolates and set into relation to reference strains by a deep sequencing approach.

## METHODS

**Human samples:** 19 Swiss human *F.t.holarctica* isolates from 2008 to 2014 belonging to the national reference repository for tularemia.

**Animal samples:** 15 *F.t. holarctica* isolates from 2012 to 2014 from free-ranging and zoo animals throughout Switzerland. One isolate originates from 1998.

**Tick samples:** In 2012, a tick “confirmation-survey” was conducted on 6 previously known *F.t.holarctica* positive sites (national tick survey of 2009<sup>1</sup>), collecting another 14’446 ticks which were sorted into pools of 10 nymphs or 5 adults. Pooled ticks were homogenized and DNA was extracted. Positive samples were identified by PCR.

11 *F.t. holarctica* strains were successfully isolated from tick pools by cultivation.

**Next generation sequencing:** NGS deep sequencing was performed with a Illumina HiSeq 2000 System. The mean coverage rate was >2000 fold for all samples. Preprocessing of the reads was performed with *SAMtools*. *Bowtie2* was used for mapping the reads against the FTNF002-00 reference strain. Variants were called by *freebayes*. SNP quality filtering and generation of SNP pseudosequences was done by in house R scripts. Pseudosequences were aligned with *clustalW* and trees were build with *MrBayes*.

## RESULTS

In accordance with previous studies<sup>2</sup> the genome-wide SNP analysis revealed that the majority of the Swiss *F.t. holarctica* isolates (41/46) belong to the Franco-Iberian sub-lineage B.FTNF002-00. Two free-ranging animals, a zoo animal and two human isolates were assigned to the north eastern B.13 sub-lineage (Figure 1). Compared to the reference strain FTNF002-00, the Swiss isolates of the Franco Iberian lineage show 173 SNPs whereas the 5 isolates which are more closely related to the B.13 lineage show 673 SNPs. Regarding the topology of the phylogenetic tree, the Swiss B.FTNF002-00 lineage shows 4 major clusters on a 13< x <23 substitution per site level. Within these clusters the geographical as well as the host origin are interspersed. On a higher similarity level (1< x <4 substitution per site), clustering of isolates from the same geographic region can be observed.

## DISCUSSION

- Two human and two isolates from free-ranging animals can be assigned to the north-east European B.13 lineage. Since these strains were not collected in the vicinity of the Swiss border an infection outside of Switzerland is rather unlikely.
- The heterogeneity of the clusters in regard to the host origin of the isolates confirms the role of ticks as important zoonotic vector between wildlife animal and human. This observation is in agreement with the coincidence of a high prevalence of *F.t.holarctica* in ticks and the elevated number of human tularemia cases in the north eastern part of Switzerland.
- Based on our data there is evidence that the 173 SNPs within the Swiss tick population allow to address epidemiological questions on a micro-geographic scale.

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